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GENETIC EVOLUTIONARY PROCESSES IN CREPIS

PROFESSOR E. B. BABCOCK, DR. G. L. STEBBINS, JR., AND DR. J. A. JENKINS
UNIVERSITY OF CALIFORNIA

I. INTRODUCTION

DURING the past quarter century the development of evolutionary theory has been marked by wide divergence in the opinions expressed by different authors concerning the *nature of the genetic processes* which make evolution possible. The development of the theory of the gene, and the dearth of information on other types of genetic change than gene mutation and their roles in evolution, led Morgan (1916; 1926; 1932) to assume that gene mutations alone were to be considered as important. The mathematical approach to the problems of balance between mutation rate, natural selection and population size, as developed by Fisher (1930), Haldane (1924–32) and Wright (1931), also dealt with gene mutations only. At any rate there was no discussion by them of any other type of genetic change than gene mutation.

Haldane (1932) recognized six classes of intraspecific hereditary differences, due to: (1) changes in extranuclear factors; (2) a change in a single Mendelian factor or gene; (3) changes in several genes; (4) structural changes in chromosomes causing differences in the location of genes but without loss or addition of any genes; (5) loss and duplication of genes through loss or addition of parts of chromosomes or whole chromosomes from the normal complement of the species; (6) addition of one or more whole sets of chromosomes. He points out that

all hereditary differences that have been thoroughly investigated seem to fall into one or more of these six classes. For the purposes of the present discussion, however, the types of genetic difference which are of general importance in evolution are much fewer. Of Haldane's six classes of genetic change, (1) and (6), on the basis of present knowledge, may be set aside as of relatively small importance for the general problem of evolution. Regarding extra-nuclear factors, we agree with Goldschmidt (1940, p. 250) that for the present the role of the cytoplasm, in so far as it is independent of chromosomal control, may be neglected. As for polyploidy, it has been pointed out by one of us (Stebbins, 1940*a*) that this is an important means of increasing the variability and the geographic distribution of species complexes in plants, but its role in evolution is secondary to that of the processes leading to new diploid species. Numbers (2) and (3) differ only as to the number of genes concerned, *i.e.*, just one basic type of genetic change is involved, *viz.*, gene mutation. Numbers (4) and (5) also involve one general type of genetic change, *viz.*, gross structural changes in the chromosomes, although there are in addition those intra-specific differences due to loss or addition of a whole chromosome. But the latter type of difference may also be set aside as of minor importance in evolution because of the weaker viability and lowered fertility resulting from the condition of unbalance in the chromosome set. We are primarily concerned, therefore, with the relative importance in evolution of two distinct categories of genetic change: gene mutations and gross structural changes in the chromosomes.

Dobzhansky (1941) distinguishes clearly between these two categories of genetic change and shows conclusively, in our opinion, the importance of both categories in the origin of species. He states (*op. cit.*, p. 21): "Mutational changes fall, consequently, into two large classes: those presumably caused by chemical alterations in the individual genes (mutation proper, otherwise known as point

mutation, transgenation, or genovariation), and those of a grosser structural kind, involving physical destruction, multiplication, or spatial rearrangement of the genes (chromosomal aberrations)."

The existence of a minor category of genetic changes, known as position effects, which simulate gene mutations but which are due to changes in the spatial arrangement of certain genes, has given rise to considerable controversy concerning the nature of genes. Goldschmidt (1938, 1940) has taken the stand that the concept of the gene must be abandoned. But, as has been pointed out by Dobzhansky (1941, p. 110), such an extreme view assumes that there are only two possible alternatives, viz., either the genes are separate corpuscular entities or they do not exist; whereas there are other concepts of the nature of genes which are just as logical and consistent with the facts of Mendelian inheritance. We agree with Dobzhansky (*op. cit.*, p. 111) that: "No matter which of these possibilities, if any, will prove true, the existence of genes is as well established as that of atoms and molecules in chemistry." And the recognition of gene mutations as a definite category of genetic changes is fully justified.

Furthermore, in connection with position effects, it is well known that the evidence for their occurrence has been restricted to *Drosophila;* and even here "the value of the position effects for the morphological differentiation of a species has been over-estimated" according to Gustafsson (1940). In plants position effects are practically unknown. No evidence of their existence has been reported in *Zea, Datura, Pisum* or *Crepis,* plant genera in which research on chromosome changes has been extensive. As far as we know the only evidence of a position effect in plants was reported by Catcheside (1939). This concerns merely the degree of pigmentation of the flower buds which is conditioned by a certain gene. Other cases of position effect in plants may come to light; but at present there is no evidence that they have been of importance in evolution.

In plants at least it now appears that gross structural changes in the chromosomes have been of evolutionary importance mainly by producing *intraspecific sterility barriers.* These barriers lead to interspecific discontinuity and, combined with morphological and physiological divergence, lead to speciation. The latter types of differentiation within and between plant species depend mainly, if not wholly, upon gene mutations. The results of the *Crepis* investigations are in agreement with this inference.

Goldschmidt (1940) takes just the opposite point of view. He concludes that Mendelian variations are of importance *only* for intraspecific differentiation and that their evolutionary role stops short at that point ("microevolution"). For the origin of new species he invokes "systematic" mutations capable of producing both interspecific sterility and morphological differentiation ("macroevolution"). His concept of the mechanism of these systemic mutations is found in chromosome changes. He states (p. 206): "A systemic mutation (or a series of such), then consists of a change of intrachromosomal pattern. This is what is actually found taxonomically (the bridgeless gap) and cytologically. Whatever genes or gene mutations might be, they do not enter this picture at all. Only the arrangement of the serial chemical constituents of the chromosomes into a new, spatially different order; *i.e.,* a new chromosomal pattern, is involved." It is worthy of note that all the cogent evidence, cited by Goldschmidt in support of his *macro*evolution hypothesis, is found in animals and that he assumes (p. 394) that the same line of reasoning would apply to plants. In our opinion this is a dangerous assumption, since we are aware of no supporting evidence from plants. On the other hand, some of the "best" evidence used in support of his *micro*evolution hypothesis is taken from *Crepis* and we believe that the facts do not support the hypothesis. In short, the point of view taken in his book is widely at variance with the sum total of evidence from the *Crepis* investigations. It

is hoped that a brief summary of this evidence and the conclusions which we have based thereon may serve to clarify the situation.

Before presenting the evidence from *Crepis,* however, the origin of interspecific sterility requires further consideration. In the preceding paragraph it was stated that, in plants at least, the main evolutionary role of structural changes in the chromosomes has been in creating sterility which may lead to interspecific discontinuity. By this we do not wish to imply that this is the only genetic process that can generate interspecific sterility. On the contrary, evidence will be submitted which shows that *gene mutations also* can lead to an effective physiological barrier between species (see p. 354). Incidentally, this evidence indicates that the development of isolating barriers by means of gene mutations may accompany, as well as follow, the accumulation of the gene complexes which determine the morphological differences between the species.

A general review of all the *Crepis* literature has been prepared by the senior author (Babcock, 1941). For purposes of the present paper we shall categorically summarize the more important evidence on evolution in *Crepis* in very brief form and then state our conclusions concerning the genetic processes which made this evolution possible. The original data on which this summary is based will be found in the literature cited at the end of this article.

II. The Genus Crepis

Crepis is a genus of the tribe Cichorieae of the great Compositae family. The best-known large, closely related genera are *Lactuca* and *Hieracium.* The genus consists of 196 species, of which 113 have been grown at Berkeley and examined cytologically. Many of these have been used in cytogenetic investigations. The genus is distributed in Eurasia, Africa and western North America; and the center of origin and distribution was evidently in western Asia. All the evidence from com-

parative morphology, geographic distribution and cytogenetics is consistent with the concept that this genus, as now delimited, is a natural group, *i.e.*, all species of *Crepis* have descended from a common ancestral stock. Yet among these species we find a remarkable range of morphological types, a significant series of chromosome numbers and chromosome types, and various degrees of genetic homology. Furthermore, the research on this group of more or less closely related species, which has been carried on in this country and in Russia for the past twenty years, has brought to light an array of evidence which is outstanding in its significance with respect to the genetic processes which have been responsible for evolution within the genus.

1. *Morphological Evidence of Evolution*

The morphological types range from primitive to advanced on the basis of criteria which are widely accepted among plant systematists as of general significance. The most primitive have a perennial root and woody caudex; large, lyrate or nearly entire leaves; tall, robust stems or, in alpine species, scapiform stems; few large flowerheads; the involucre poorly differentiated into outer and inner series of bracts, or with large outer bracts, and the inner bracts unchanged at maturity; large florets; large, fusiform or coarsely beaked achenes; and coarse, stiff pappus-bristles. In marked contrast, the most advanced diploid species are small, very precocious annuals; with slender, ephemeral root and caudex; small, often dissected leaves and low, slender stems bearing many small heads; the involucre has few very small outer bracts and the inner bracts are specialized by cortical thickening; the florets and achenes are very small and the achenes have a very fine beak bearing extremely fine, soft pappus. Between these two extremes there are series of groups of species exhibiting various degrees of advancement or specialization; and within many of these groups there is a lesser range from more primitive to more advanced species.

2. *Cytological Evidence of Evolution*

a. The chromosomes of related genera. Certain genera, particularly *Dubyaea* and *Prenanthes,* contain some species which are morphologically more primitive than the most primitive *Crepis* species. In fact, they are among the most primitive types in the tribe Cichorieae. These species have the haploid numbers 8 and 9, which appear to be the primitive numbers of the tribe. The karyotype, or visible pattern of the chromosomes, is similar in all these most primitive relatives of *Crepis,* being characterized by the large size and symmetrical form of most of the chromosomes. (By symmetrical form is meant the nearly median location of the centromere, *i.e.,* the spindle-fiber-attachment body, thus making the two arms of the chromosome nearly equal in length.) Such a karyotype is in sharp contrast with those of most *Crepis* species, in which the individual chromosomes have been so transformed that the centromere is much nearer one end, thus making one arm much shorter than the other.

b. Chromosome numbers in Crepis. There are 16 species which are polyploids of one sort or another; but these will not be considered here, since the processes involved in their origin are considered to be of secondary evolutionary importance. The known haploid numbers of the true diploid species are distributed as follows:

Haploid number	Number of species
3	3
4	57
5	19
6	14
7	3

Apparently $n = 3$ represents an end-point in an evolutionary trend marked by reduction in chromosome number. The small group with $n = 7$ is not as primitive as the 6-paired species and requires a special hypothesis, involving intergeneric hybridization, to explain its origin. The remaining diploid species have $n = 6$, 5 or 4; and of these the most primitive numbers are 6 and 5. This is based on the fact that the most primitive species of *Crepis,* as determined from plant morphology, also have

the most nearly symmetrical chromosomes. Of the nine most primitive species in the genus, six have $n = 6$, and three have $n = 5$ chromosomes. Concerning the distribution of chromosome numbers in the genus, there are 96 diploids, of which 34 per cent. have either 5 or 6 as the haploid number and 60 per cent. have 4, the remaining 6 per cent. being the 3-paired and 7-paired species. When the chromosome numbers of all the species in the genus are known, the proportion of 4-paired species may be somewhat larger. But this will not affect the conclusion that 5 and 6 are the primitive numbers in *Crepis*, because all these probably 4-paired (or possibly 3-paired) species are more advanced on morphological grounds than the most primitive 5-paired species. It simply means that reduction in chromosome number has accompanied, or been followed by, differentiation through morphological reduction and specialization in the plants.

c. Karyotypes of diploid Crepis species. The salient facts concerning the number, symmetry and relative length of the chromosomes in the diploid species of *Crepis* are shown in Fig. 1. The small group of 7-paired species are not included for reasons stated above. These idiograms were drawn from measurements made on camera lucida drawings of uniform magnification of mitotic metaphase plates in root-tip cells. No pretense is made as to a high degree of accuracy; but the idiograms are sufficiently close to the actual lengths to indicate faithfully the trends of karyotype evolution.

It will be noted that *C. kashmirica* has the longest total length of chromosomes of all the species that have been studied cytologically. Actually its chromosomes approach more nearly than those of the other species shown toward the equi-armed type characteristic of the primitive 8- and 9-paired species of related genera. In this respect *kashmirica* is similar to several other primitive 6-paired species of *Crepis*. The most advanced 6-paired species is *C. Mungierii*, a low-growing, small-headed perennial, endemic in Crete. Its total chromosome length is only 46 per cent. of the total length of the chromosomes in

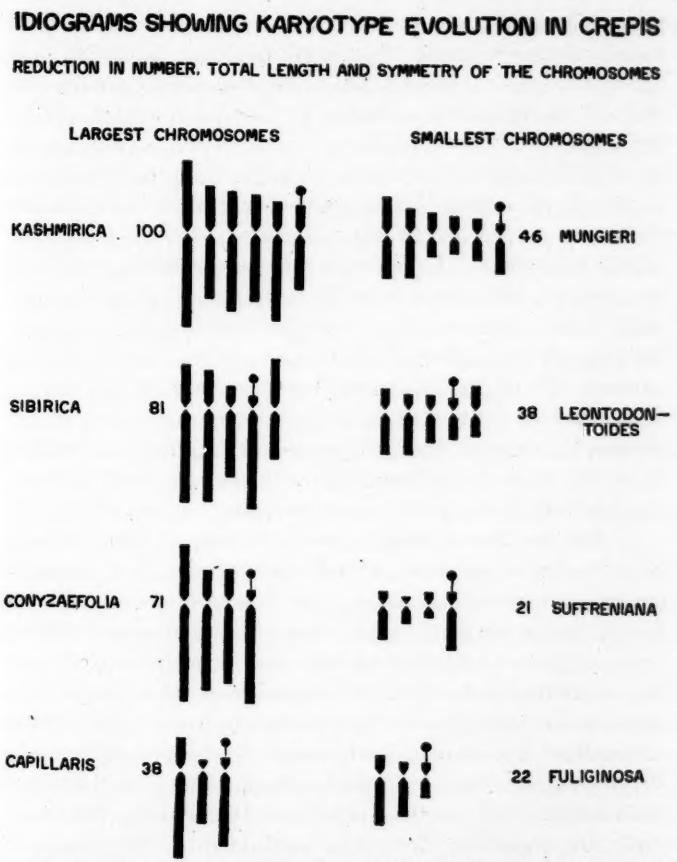

FIG. 1. These diagrammatic representations of the actual chromosomes show the karyotypes of the species with longest and shortest total chromosome length in each of the four number classes, $n = 6, 5, 4$ and 3. The reduction in symmetry is emphasized by placing the spindle-fiber constrictions on a common base line in each number class. Using the total length of *kashmirica* as a base of 100, the proportional total length of each idiogram is shown by the number between the name of the species and its idiogram. These idiograms are not perfectly accurate as to the absolute size of the individual chromosomes, but they are sufficiently accurate for purposes of the present discussion.

C. kashmirica, although its degree of symmetry is higher, owing to the nearly equi-armed condition of the 3 smallest chromosomes, a rather unusual feature in *Crepis*. The

other 6-paired species form a more or less continuous series between these two extremes (*cf.* Babcock and Cameron, 1934; Babcock and Jenkins, unpublished).

Similar comparisons hold in general for the species with greatest and least total length of chromosomes in the 5-paired species, *C. sibirica* and *C. leontodontoides;* in the 4-paired species, *C. conyzaefolia* and *C. Suffreniana;* and in the 3-paired species, *C. capillaris* and *C. fuliginosa.* It will be noted that the two 5-paired species shown have one equi-armed member in the karyotype. Such a member is characteristic of all the 5-paired species; whereas its absence is characteristic of the 4-paired and 3-paired species. Furthermore, the other members of the karyotype have a general similarity throughout these three number classes. Such a degree of similarity suggests that the important steps in reduction in chromosome number were infrequent or rare events.

d. Parallel trends in Crepis evolution. There is a certain degree of parallelism between reduction in chromosome *number* and reduction in size and life cycle of the plant. All the 6-paired species are primitive perennials; some of the 5-paired species are less primitive and a few are annuals; a few of the 4-paired species are fairly primitive perennials, but more of them are less primitive and many are annuals; while all three of the 3-paired species are reduced or specialized annuals. But the great majority of the species are either 5-paired or 4-paired; and *within each of these two groups* there is a general trend toward reduction in size and life cycle of the plants. Therefore, the parallelism between reduction in chromosome *number* and reduction in the plants does not indicate a cause-and-effect relation between the two. On the contrary, as will be shown below, the two trends are the results of entirely different processes of genetic change. The events leading to reduction in chromosome number as stated above seem to have occurred only rarely, whereas morphological differentiation appears to have gone on continuously.

Reduction in chromosome *size,* on the other hand, is of

general occurrence in all four number classes and in the several major taxonomic groups within the genus. There is a close parallelism (Babcock and Cameron, 1934), with a few exceptions, between reduction in size of the chromosomes and progressive reduction in size of the plant and specialization of parts as well as length of the life cycle (long-lived perennials, short-lived perennials, biennial-monocarpics, slow-growing annuals, precocious annuals). Delaunay (1926), working on *Muscari*, first called attention to such a parallelism, but he made no suggestion as to the cause of reduction in chromosome size. This problem is discussed below (p. 357).

Another striking parallelism has long been recognized in *Crepis* (Hollingshead and Babcock, 1930; Babcock and Navashin, 1930; Babcock and Cameron, 1934; Babcock, 1934). This has been summarized (Babcock, 1936) in part as follows. It is certain that morphologically similar species have similar chromosomes and, conversely, close similarity of the chromosomes has often proved to be a helpful clue to a closer degree of relationship than was at first surmised from superficial gross morphology of the plants. Thus it has been possible to work out with greater accuracy the classification of species into groups and to ascertain phylogenetic relations. Certain groups of closely related species have been shown to possess almost identical karyotypes without any notable structural differences in their chromosomes. This shows that close similarity in the chromosomes is usually correlated with close taxonomic relationship in *Crepis*. It also indicates that the genetic evolutionary processes involved in speciation within these groups are processes which are not accompanied by any considerable changes in the chromosomes. The species in such a group were assumed to possess closely homologous chromosomes and to have become differentiated through gene mutations. The results of later investigations on certain of these groups of species have shown that this assumption was warranted.

It should be emphasized, however, that karyotype similarity is not the cause of morphological resemblance in

the plants. Its cause is found in the similarity of very many of the genes which the species possess. In other words, karyotype resemblance *alone* will not serve as a taxonomic criterion in *Crepis* because some sections which are widely separated morphologically have similar karyotypes. But this only strengthens the inference that gene mutations have been of great importance in speciation. Apparently it has been equally important in the differentiation of some of the primitive genera which are closely related to *Crepis,* for example, *Dubyaea, Prenanthes* subg. *Nabalus* and *Soroseris* (Babcock, Stebbins and Jenkins, 1937; Stebbins, 1940*b*) since the karyotypes in these widely different genera are indistinguishable.

3. *Cytogenetic Evidence on Evolution of Diploid Species*

a. Hybrid viability and fertility. Interspecific hybridization has been carried out on an extensive scale, both in Russia and the United States. In our own work, between 1920 and 1939, seed was obtained from 206 interspecific crossings involving 59 different species. In general, hybrids between species which are less closely related, as judged from the morphology of the plants, tend to be weak and sterile or, if vigorous, to be sterile or of very low fertility; whereas hybrids between more closely related species tend to be vigorous and more or less fertile. But in the latter the fertility is low, even when the two species have the same chromosome number, if they differ much in the structure of their chromosomes.

b. Meiosis in interspecific hybrids. As a general rule meiosis is regular in the species of *Crepis* which have been investigated (*cf.* Babcock, 1941), with bivalents formed regularly at first metaphase. In the hybrids between a few closely related species the meiotic behavior of the chromosomes has been as regular as in the parents. But in most interspecific *Crepis* hybrids there is more or less failure to form pairs at metaphase. There usually is some degree of pairing, however, and, assuming that pairing depends upon genic homology, the evidence on meta-

phase pairing in interspecific hybrids supports the conception that the species of *Crepis* had a common origin and are still more or less similar in genic constitution.

Structural hybridity has been discovered in several *Crepis* species through the investigation of meiosis in interspecific hybrids. Müntzing (1934) studied a hybrid between *C. divaricata* and *C. Dioscoridis,* both being 4-paired species, but classified in widely separated sections of the genus. An average of only 1.8 bivalents was found at first metaphase in pollen mother cells which indicates considerable lack of homology between the chromosomes of the two species. At the same time fragments and chromatin bridges were observed in many cells, from which Müntzing concluded that the chromosomes of the two species have certain homologous segments. Müntzing emphasized the idea that chromosome relationships such as these provide a mechanism capable of generating new chromosomal alterations of evolutionary value. The origin of new chromosome types through such a mechanism, in natural interspecific hybrids, has doubtless played a certain role in karyotype evolution and in the origin of species in *Crepis* (see p. 360). But this is a secondary process as compared with the genesis of gross chromosomal changes within a species. The existence of homologous segments in *C. divaricata* and *C. Dioscoridis* certainly indicates that they had a common ancestry and that their evolution has been accompanied by certain structural changes in the chromosomes. How did the first chromosomal alterations arise? Direct evidence is given below (p. 350) showing how this occurs within a species.

More convincing evidence on the relation between partial chromosome homology and reduction in chromosome number has been discovered by Miss Sherman (unpublished), who studied meiosis in hybrids between *C. Kotschyana* ($n=4$) and six closely related species in all of which $n=5$. *C. Kotschyana* had been classified along with these 5-paired species on the basis of close morphological similarity. Strong indications of chromosome

homology were found in all six hybrids. At first metaphase in the pollen mother cells, besides univalents, bivalents and trivalents, there were sometimes quadrivalents or higher polyvalents. And at first and second telophase all six hybrids showed one or two chromatin bridges and one or more fragments in a certain proportion of the cells examined. This strongly confirms the assumption that the 4-paired *Kotschyana* was derived from the same ancestor as its six 5-paired relatives. Still stronger evidence that chromosomal transformation leads to reduction in chromosome number and to karyotype modification, through changes in the individual chromosomes, is presented in the following section.

c. Karyotype analysis. H. A. Tobgy (1941) has investigated *C. neglecta* ($n=4$), *C. fuliginosa* ($n=3$), both F_1 and F_2 hybrids, and certain forms found in nature. The study of meiosis in F_1 hybrids revealed definite evidence of the existence of homologous segments in the chromosomes of the two species. For convenience in description the chromosomes of *neglecta,* as shown from left to right in Fig. 2 are designated A, B, C and D; those of *fuliginosa* as A, B and D.) The A and D chromosomes of *neglecta* are structurally similar to the A and D of *fuliginosa,* except for an unequal reciprocal translocation. The B chromosome of *fuliginosa* is mostly homologous with the B of *neglecta,* but it contains also the essential material of the C of *neglecta.* One arm of the *neglecta* C chromosome and its centromere are absent from the *fuliginosa* complement. These facts show that the 3-paired *fuliginosa* was derived from *neglecta,* or from a common 4-paired ancestor, through a process involving chromosome interchange and resulting in reduction from 4 to 3 pairs of chromosomes as well as in marked change in the karyotype. This direct evidence on the origin of a 3-paired species from a 4-paired ancestor proves that structural changes in the chromosomes are the processes involved in karyotype evolution in *Crepis.* But, as was pointed out (p. 347), karyotype evolution *per se* is not the cause of speciation. The importance of structural

changes in the chromosomes in the origin of species is due to the fact that they create interspecific sterility.

Because of the structural differences in the chromosomes of *neglecta* and *fuliginosa,* the F_1 hybrids were highly sterile. However, several intermediate forms, suspected of being of hybrid origin, have been collected in northeastern Thessaly where the two species were known to have come into contact. By persistent effort Tobgy

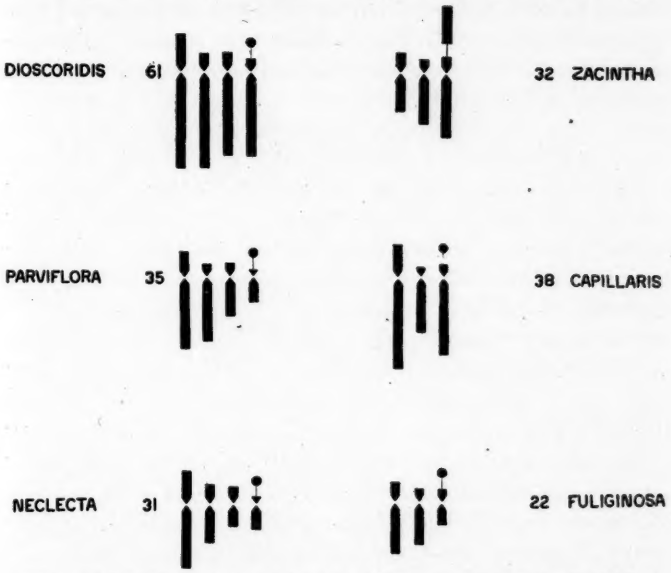

FIG. 2. Karyotypes of three 3-paired *Crepis* species and their closest 4-paired relatives. Proportional reduction in total length of the chromosomes, indicated by numbers referred to a base of 100, is paralleled by reduction in size and specialization of parts in both the 4-paired and 3-paired series.

was able to obtain F_2 and backcross progenies totaling 64 plants, among which were both parental and F_1 types as well as numerous new forms with chromosome numbers ranging from 6 to 11. One of the F_2 segregants was similar in morphology and karyotype to one of the wild intermediate forms which was about 70 per cent. fertile. It has a *neglecta* karyotype but one or more chromosomes carry *fuliginosa* segments, which explains the presence of

certain *fuliginosa* characters. The occurrence of a duplicate of this wild form among the F_2 segregants provides the best clue to the method of origin of the intergrades occurring in nature. In addition to the above form, which was produced by two successive crossovers in the same chromosome arm, several new forms with changed karyotypes, resulting from single crossing over, were found among the F_2 progeny. Again interspecific hybridization is seen to be one process by which potential new species may originate in nature; but it is strictly secondary to the intraspecific chromosomal alterations which cause hybrid sterility.

4. *Experimental Modification of the Karyotype*

a. Results of treating seeds with x-rays. Spontaneously occurring alterations in the chromosomes of *C. tectorum* had been observed by Navashin (1931*a*), and this led to their production under controlled conditions. Experimental alteration of the chromosomes in *Crepis* has received considerable attention from Navashin (1931*b*, 1932), Levitsky (1935), Korjukaev (1940) and other Russian investigators. The species used were *C. tectorum* ($n=4$) and *C. capillaris* ($n=3$). The work was extensive, involving the cytological study of hundreds of seedlings. Levitsky (1940) reported that among 491 seedlings examined 74 or 15 per cent. showed structural chromosome changes, including translocations, inversions and duplications. Translocations were the most frequently occurring type of structural change and most, if not all, of these were reciprocal translocations between nonhomologous chromosomes. Thus the type of change, assumed by Müntzing, Sherman and Tobgy to have been responsible for the homologous segments in certain species, is the same type that occurs most frequently when the cells of somatic tissues are treated with x-rays.

b. The genesis of intraspecific sterility. Gerassimova (1939) has succeeded in meeting again[1] the demand of

[1] Dubinin (1934) was the first to make a comparable achievement, using *Drosophila melanogaster*. The partial sterility of aneuploid forms, such as the *Datura* trisomics, is less significant for evolution.

Bateson (1922): "The production of an indubitably sterile hybrid from completely fertile parents which have arisen under critical observation from a single common origin is the event for which we wait." Using *C. tectorum*, she obtained, among the progeny from x-rayed material, two plants which were homozygous for different reciprocal translocations. One involved the A and D chromosomes and the other, the B and C chromosomes. Both were morphologically identical with normal *tectorum* and just as self- and cross-fertile. By crossing them F_1 hybrids were obtained in which each of the four chromosome pairs differed structurally; but the plants differed from normal *tectorum* only in lowered fertility. Selfing these hybrids produced progeny comprising plants with normal karyotypes as well as all the possible combinations of heterozygous and homozygous translocations, including one which was homozygous for translocations in all four chromosome pairs and which was called *C. nova I*. This karyotypically new strain was morphologically identical with normal *tectorum* and equally viable and fertile. But, when *C. nova I* was crossed with normal *tectorum*, the F_1 hybrids were only 30 per cent. fertile when selfed and but slightly more when open-pollinated.

The study of meiosis in these hybrids revealed chromosome behavior in full accord with expectations, including formation of quadrivalents involving normal chromosomes and their translocated mates; and it is assumed that all gametes except those resulting from alternating distribution of the chromosomes to the poles are inviable. A certain amount of fertility, however, might be due to combinations of new crossover types of chromosomes. It is also suggested that part of the sterility may be due to other causes. Whatever the true nature of the sterility may be, there certainly exists a highly efficient mechanism causing physiological isolation between two constant forms of the same species. It is also very probable that crossing over between homologous segments will produce further structural changes resulting in additional sterility. At any rate, it is beyond question that there has

been created here "a situation characteristic of a hybrid between two genuine species." And, although *C. nova I* is still morphologically indistinguishable from the original *C. tectorum* "accumulation of mutational changes should undoubtedly lead in future to such distinction."

Although not suggested by Gerassimova, it is highly probable that all her anticipated new mutations, leading to morphological and physiological differentiation, would be gene mutations. It should also be recognized that the initial hybrid sterility, which is set up by the translocations in *C. nova I*, is only partially characteristic of interspecific sterility in general. That such sterility would be built up, however, along with morphological and physiological diversity, by ensuing gene mutations is clearly indicated by the evidence on partial intersterility in hybrids between species which differ only in Mendelian variations (see p. 357).

c. Aging of seeds as a means of inducing chromosome changes. It has been clearly demonstrated by Navashin, Gerassimova and Belajeva (1940) and others that chromosome transformations, similar to those induced by x-rays, are produced in considerable numbers in normal dormant seeds when stored under conditions of high humidity or high temperature. They also occur "spontaneously," though more rarely, in seeds stored under ordinary room conditions. In view of the endless variety of conditions under which seeds in nature may await a suitable opportunity for germination, it seems probable that here is another natural cause of this type of genetic evolutionary processes in plants.

5. *Evidence that Interspecific Sterility Can Originate through Gene Mutations*

Some of the groups of closely related *Crepis* species with identical karyotypes (see p. 347) have been investigated cytogenetically in order to ascertain the nature and extent of their hereditary differences. One such group consists of three endemic species, isolated on the Madeira and Canary Islands, *C. canariensis,*

C. Noronhaea and *C. divaricata,* together with two subspecies of *C. vesicaria* viz., *andryaloides* and *taraxacifolia.* The latter subspecies was evidently introduced into Madeira by early colonists. It is fully naturalized and has spread around Funchal and along a trail to the north side of the island, where it met one of the endemics, *andryaloides.* The two have hybridized and produced a swarm of more or less fertile intermediate forms. For this reason *andryaloides* is considered a subspecies of *vesicaria;* but morphologically it is just as well differentiated as the other three endemics. Although there is a general similarity among all five entities, yet they differ in a great many morphological features affecting all parts of the plant. In the F_1 hybrids between them by far the greater number of these differences appeared to be conditioned by a large number of multiple genes.

In his experimental hybrids between the five entities Jenkins (1939) found the meiotic behavior of the chromosomes to be almost perfectly regular, just as in the parents. He states: "The cytological evidence strongly indicates that the five entities have a similar arrangement of genes in the various chromosome types. In other words, there have been no large duplications, translocations, or other rearrangements that in any way interfere with normal meiosis." From this it may be inferred that most, if not all, of the genetic variations between the five entities are the result of gene mutations. Nevertheless, it was found that the average fertility of the F_1 hybrids, as indicated by percentage of seed setting with open pollination, was 25–50 per cent. The least fertile hybrids had only 1–2 per cent., and the most fertile, 50–75 per cent., as compared with nearly 100 per cent. in all the parents. Thus interfertility between all five has been definitely though not completely reduced, evidently as a result of gene mutations. For taxonomic purposes the fact of geographic or ecologic isolation warrants the recognition of *C. divaricata, C. Noronhaea, C. canariensis* and *C. vesicaria* as species; whereas *andryaloides* and *taraxacifolia* may be considered as subspecies of *C. vesicaria* because their vigor-

ous hybrids are apparently sufficiently fertile to break down their morphological distinctness.

Goldschmidt (1940, p. 159) finds here further evidence to support his hypothesis that Mendelian mutations can not function in the origin of species. But we find, in this group of closely related species, not only good morphological distinction and differences in many genes, but that as a result of gene mutations there exists a partially developed internal isolating mechanism. Such isolation is accepted by Clausen, Keck and Hiesey (1939) as a criterion for recognizing ecospecies which usually correspond to taxonomic species. From evidence such as this it is very clear that in *Crepis,* at least, gene mutations are an evolutionary process of major significance. For they not only build up intra- and inter-specific sterility but, since they are omnipresent, they must continually supplement and extend the other genetic processes concerned in the development of new species.

III. The Genetic Processes Causing Evolution in Crepis

We believe that the evidence presented above affords convincing proof that both gene mutations and structural changes in the chromosomes are primary causes of evolution in *Crepis.* The bearing of this evidence may be more fully clarified, however, by reviewing the roles played by these two categories of processes.

1. *The roles of gene mutations.* Two different aspects of *Crepis* evolution are found to depend upon gene mutations, *viz.,* morphological and physiological differentiation and accumulation of intra- and inter-specific sterility; while a third, reduction in chromosome size, may also be attributed to gene mutations.

a. Differentiation. There are numerous polymorphic species of *Crepis,* for example, *capillaris, tectorum, Dioscoridis* and *foetida,* in which genetic experiments have shown that intraspecific variations, both morphological and physiological, are Mendelian in character. There are

also groups of very close species with identical karyotypes, between which numerous Mendelian differences exist, for example, the group of island endemics discussed above and the *foetida, eritreënsis, Thomsonii* group (*cf.* Babcock, 1941). There can be no doubt that gene mutations have caused such gradual accumulations of differentiating Mendelian variations. Furthermore, this process of gene mutation is always going on and is ready to play its part whenever circumstances favor differentiation and discontinuity. When intraspecific isolation is accomplished through changes in chromosome structure, gene mutations will soon begin the process of differentiation, if they have not already done so.

b. Accumulation of intersterility. In addition to Jenkins's island endemics, there is other evidence from *Crepis,* showing that gene mutations cause intersterility. In the *foetida, eritreënsis, Thomsonii* group, mentioned above, some of the first interspecific hybrids studied (Babcock and Cave, 1938) were highly fertile, while others were more or less sterile. This low fertility, because of technical difficulties, could not be considered as significant. More recently, however, additional evidence (unpublished) has been found which shows that, when certain strains are used, subspecific crosses within *C. foetida* are highly sterile. It is safe to assume, therefore, that the interfertility situation in this group of three species is comparable to that found by Jenkins in the island endemics. This is mentioned here because the *foetida* group was also cited by Goldschmidt in support of his "microevolution" hypothesis.

c. Reduction in chromosome size. The parallelism between the general trend toward reduction in chromosome size and progressive reduction in the plants and their life cycles throughout the genus was mentioned above (p. 346). This case of parallelism in evolutionary trends was first reported by Babcock and Cameron (1934), but no assumptions were made regarding its possible cause. Although no direct evidence regarding the cause of chromosome

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shortening is yet available in *Crepis,* there is plenty of evidence showing that the morphological and physiological variations in the plants depend upon genic differences. It would seem reasonable, therefore, to assume that this trend in reduction of chromosome size is also a visible effect of genotypic changes. The loss of large segments of euchromatin, as the cause of general reduction in chromosome size, is of course not to be considered because of the known deleterious effects of such losses. The loss of segments of heterochromatin may have caused reduction in length in some cases. This certainly is true in the case of *C. fuliginosa.* As stated above (p. 350) *fuliginosa* lacks parts of the C chromosome of *neglecta,* including the centromere, and Tobgy found that this missing portion is largely composed of heterochromatin. That this is the chief cause of chromosome shortening, however, seems unlikely. Heterochromatin in plants is not necessarily "inert," although absence of genes is often assumed. In Drosophila, "inert" is certainly a misnomer (Schultz, 1936) but the situation in plants remains to be determined.

A direct relation between chromosome size and the metabolism of the plant has been demonstrated by Pierce (1937). Excess and deficiency of phosphorus, as compared with a complete culture solution, caused corresponding increase and reduction in length, width and volume of the chromosomes in a species of *Viola.* Variations in available soil nutrients under natural conditions might cause some differences in chromosome size, but under controlled conditions of culture this variable would be eliminated. All *Crepis* cultures, used in cytogenetic investigations, have been grown under conditions as nearly uniform as possible. Pierce (*op. cit.*) also reports that excess phosphorus caused only slightly more vigorous plant growth, whereas deficiency produced a marked dwarfing effect upon the whole plant. This dwarfing effect and the reduced size of the chromosomes in the dwarfed plants are evidently both due to phosphorus de-

ficiency. An analogous pleiotropic genic effect could easily be imagined, *i.e.*, the same gene mutations might induce reduction in the plant and reduction in chromosome size.

In his analysis of hybrids between *C. neglecta* and *C. fuliginosa,* Tobgy found that, in contrast with the situation reported by Navashin (1934) for other *Crepis* hybrids, the chromosomes of these two species retain their characteristic width at somatic metaphase in the F_1 hybrid; *i.e.*, those of *C. fuliginosa* are always about two thirds as wide as those of *C. neglecta.* Examination in F_2 and backcross plants of "new" chromosome types resulting from crossing over in the F_1 hybrid, showed that the width of a chromosome is always uniform, even though it is composed of segments which originally differed in width. On the other hand, transferred segments, even though their width is altered, retain their original length. This shows, as Tobgy points out, that length and width are not entirely controlled by the same factors but may vary independently. In other words, the differences in chromosome size in these two *Crepis* species have not resulted from genetic changes affecting the chromosomes as a whole; hence the genotypic changes postulated by Darlington (1937, pp. 53–54) are not applicable in this case.

While it is impossible, in view of the incomplete and somewhat conflicting nature of the evidence from different plants, to suggest the exact nature of the genetic control of chromosome size, nevertheless the fact of some genetic control of size is clearly indicated. Furthermore the progressive reduction in the chromosomes, which has accompanied reduction in size and life cycle of the plants, may well depend upon gene mutations.

2. *The roles of changes in chromosome structure.* Changes in chromosome structure have been shown, in the foregoing review of evidence from *Crepis,* to have played two different roles, *viz.*, the genesis of interspecific sterility and progressive differentiation in the karyotype.

The relative importance of these roles in the origin of species will be briefly discussed.

a. Genesis of intersterility leading to speciation. On *a priori* grounds this is the only role played by changes in chromosome structure which could be of primary importance in speciation. And, since the origin of such sterility might either precede or follow extensive differentiation through the accumulation of gene mutations, the one process can not be considered any more basic than the other as a cause of speciation. This conception is advanced, however, with one reservation. There is evidence from *Crepis* indicating that certain genetic differences among the individuals of a species have a marked effect on the frequency of occurrence of chromosome changes caused by x-rays. Levitsky (1937) reported that the chromosomes in *C. capillaris* are very stable, but that among 295 plants grown from x-rayed seeds there were 25 showing deviations in chromosome morphology. These were confined to 11 out of 28 families, and two of these families had 11 of the 25 cases. Hence, he concludes, there are genetic differences conditioning structural instability and these may be an important cause of karyotype evolution. If future investigations should prove the existence of genes which condition liability to the occurrence of structural changes, then to this extent gene mutation would have to be considered a more basic process than gross chromosomal changes. For the present, however, it is sufficient to recognize both processes as of primary importance in speciation.

b. Karyotype evolution. Karyotype evolution in *Crepis* is characterized by progressive reduction in chromosome number, increase in asymmetry of the individual chromosomes and reduction in total length of the chromosomes. That the parallelism between reduction in chromosome number and reduction and specialization in the plants is coincidental has been pointed out. Evidence has been presented showing that reduction in chromosome number has been made possible by changes in

chromosome structure, principally by reciprocal translocations; whereas progressive reduction and specialization of the plants has depended entirely upon gene mutations. As far as we know the possible evolutionary significance of position effects may be disregarded. Gene mutations and structural changes in the chromosomes proceed independently but apparently fortuitously in *Crepis*, except in so far as the latter *may* depend upon the former.

The parallelism between increase in asymmetry of the individual chromosomes and progressive evolution of the species is likewise apparently fortuitous. Modification of the individual chromosomes, like reduction in chromosome number, depends on changes in chromosome structure, whereas morphological and physiological differentiation within and between species depends on gene mutations.

Reduction in chromosome size, on the other hand, and reduction in the plants can both be referred to the effects of gene mutations. This particular parallelism appears not unlikely to rest upon a common cause for both categories of phenomena.

IV. Summary

1. The primary genetic processes causing evolution in *Crepis* are gene mutations and structural changes in the chromosomes.

2. The roles of gene mutations are the production of morphological and physiological differentiation within and between species, the accumulation of intra- and interspecific sterility, and possibly the reduction in absolute size of the chromosomes.

3. The roles of chromosome changes are the genesis of intraspecific sterility leading to discontinuity and hence to speciation, and karyotype evolution through reduction in number and symmetry of the chromosomes.

4. The secondary genetic processes involved in the evolution of *Crepis* are interspecific hybridization, polyploidy and apomixis.

5. The roles of interspecific hybridization are the origin of a small number of new species, especially through amphidiploidy (*cf.* Babcock and Stebbins, 1938; Stebbins and Babcock, 1939), and the augmentation of karyotype evolution.

6. The roles of polyploidy and apomixis are a small amount of speciation combined with extensive differentiation and geographic distribution (Babcock and Stebbins, *op. cit.*).

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ON THE SIGNIFICANCE OF THE CONSTANT b IN THE LAW OF ALLOMETRY $y = bx^a$

DR. H. LUMER, DR. B. G. ANDERSON* AND DR. A. H. HERSH
DEPARTMENT OF BIOLOGY, FENN COLLEGE, AND BIOLOGICAL LABORATORY, WESTERN RESERVE UNIVERSITY

INTRODUCTION

THE law of allometry

$$y = bx^a \quad \text{..... (1)}$$

has been found to have a wide application in the study of relative growth and size relations in organisms of various types. In employing the equation, investigators have generally made extensive use of the constant a, which represents the ratio of the percentage growth rates (or per cent. rates of change) of the two parts denoted by y and x. At the same time, they have usually paid little attention to the constant b, which is the value of y when $x = 1$. In fact, this constant has been explicitly regarded by some investigators as having no particular biological significance (see, for example, Huxley, 1932, p. 4).

This failure to find any meaning or useful interpretation of the value of b arises from the fact that its numerical value depends on the units in which the parts under consideration are measured. Since these are chosen from standard, conventional sets of units (*e.g.*, the metric system) and are not related in any way to stages of development of the organism, it is not surprising that the significance becomes obscured. Moreover, as will be shown below, attempts to employ such values of b, even for purposes of comparison of different forms or organs, may for the same reason lead to erroneous conclusions.

The purpose of this study is to analyze the consequences of the dependence of the value of b on the units employed and to show how this affects its meaning and usefulness.

* Dr. Anderson's address is the Department of Botany and Zoology, West Virginia University, Morgantown, W. Va.

Relation between b and a

In a number of instances, the values of b and a for the same parts in a series of related organisms have been found to exhibit an inverse exponential relationship of the form

$$b = Be^{-ra} \quad \text{..... (2)}$$

This relataionship was first proposed by Hersh (1931) for the dorsal lobe/ventral lobe relation in various stocks of bar-eyed Drosophila, later by Hersh (1934) for certain proportions of the skulls of titanotheres, and subsequently has been found to hold in numerous other cases (*e.g.*, Hamai, 1938, 1940; Lumer, 1939a, 1940; Anderson and Busch, 1941; Clark and Hersh, 1939).

Lumer (1936) has shown that where equation (2) holds, the curves of equation (1) from which it is obtained form, on a double logarithmic grid, a family of lines intersecting at a common point with coordinates ($\log x_0$, $\log y_0$), where $\log x_0 = r$ and $\log y_0 = \log B$. Since the curves obtained by Hersh (1931, 1934) exhibit no such common intersection, Lumer concluded that equation (2) can be regarded as only a crude empirical approximation. It should be noted, however, that in some cases (Hamai, 1940; Anderson and Busch, 1941; Lumer, 1940) an approximation to a common intersection point does occur.

In a later publication, Lumer (1939b) showed that an exponential relationship between b and a is to be expected on the basis of certain dimensional features of the constants. He argued further that the relationship would generally be an inverse one (*i.e.*, $r > 0$), overlooking the fact that the value of r depends on that of b, which in turn can be changed arbitrarily by using different units of measurement.

In fact, the same set of data can be made to yield a positive, zero or negative value of r simply by choosing appropriate units. Thus, in Fig. 1, the curves are so constructed that they all intersect at the point corresponding to $x = 10$, $y = 10$. The values of b and a are in this case obviously inversely related ($r > 0$). If the unit of mea-

sure is changed to one which is ten times the magnitude of the original, the point of intersection will then correspond to $x = 1$. The values of b now are all equal, and r in equation (2) becomes zero. If the unit of measure is again increased tenfold, the values of b fall at the value of x originally denoted as $x = 100$. Here b varies directly with a.

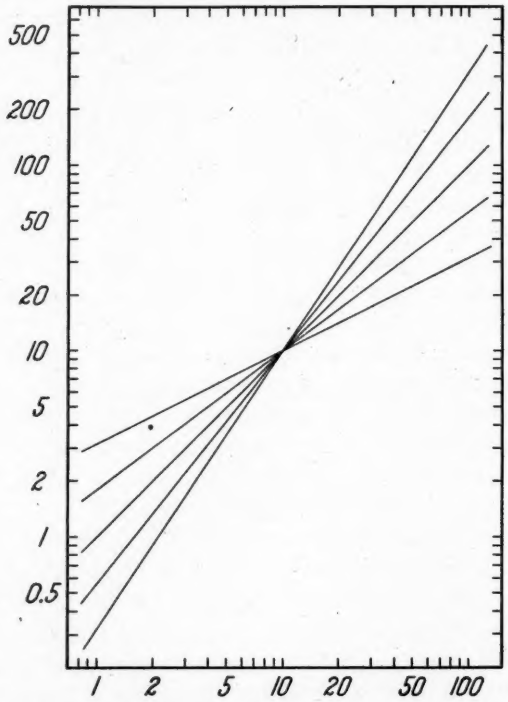

FIG. 1. Double logarithmic plot of a set of hypothetical relative growth curves constructed so as to intersect at a common point where $x = 10$, $y = 10$ (see text, page 365).

The algebraic explanation for these three cases is as follows. From (2) we obtain

$$\log b = \log B - ra$$

Since $\log B = \log y_0$, and $r = \log x_0$, where x_0 and y_0 are the

coordinates of the common point of intersection (Lumer, 1936), the above equation becomes

$$\log b = \log y_0 - a \log x_0$$

or

$$\log y_0 - \log b = a \log x_0$$

When a is restricted to positive values, it is evident that if $\log y_0 > \log b$, then $\log x_0 > 0$; if $\log y_0 = \log b$, $\log x_0 = 0$; and if $\log y_0 < \log b$, $\log x_0 < 0$. That is, $r \gtreqless 0$ as $\log y_0 \gtreqless \log b$. In short, whether the relationship is inverse or direct depends entirely on whether the value of x at the point of intersection is greater than or less than unity in the particular unit of measurement employed.

When a is negative, the reverse occurs; namely, $\log x \gtreqless 0$ as $\log y_0 \lesseqgtr \log b$. This situation might arise, for example, where the resorption of a structure is concerned.

It is thus evident that the value of r and with it the apparent character of the relationship between b and a is radically affected by the choice of units. That the relationships obtained in practice have all turned out to be inverse arises simply from the fact that the units are generally, as a matter of convenience, chosen so as to be small compared to the sizes of the structures being measured. This does not mean, of course, that the real relationships are in any way altered by merely changing the size of the unit. It shows, rather, that in order to determine the real relationships, it is necessary to find a rational unit of measure, given by the organism itself, which will eliminate the obscuring effects introduced by the use of conventional units.

The practice of selecting a unit of relatively small magnitude also explains why a relationship between b and a often occurs, even when there is clearly no indication of a common point of intersection. This is illustrated in Fig. 2, in which the curves given by Hersh (1934) for the

zygomatic width-basilar length relation in the titanotheres are extended considerably beyond the range of the data. From the figure it is clear that, compared to the *extended* range of the curves, the points of intersection do, on the whole, fall within a relatively restricted area, namely, the area covered by the range of the actual mea-

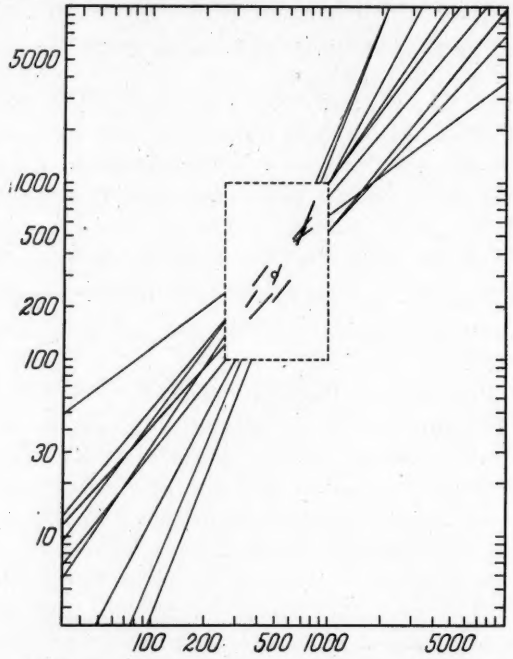

FIG. 2. Double logarithmic plot of the zygomatic width/basilar length relations in titanothere skulls from Hersh (1934). The curves are extended considerably beyond the original figure which is represented by the small rectangle. The circle in the center is the theoretical point of intersection as calculated from equation (2).

surements, enclosed within the small rectangle. For this set of curves, Hersh obtains a very sharply defined relationship between lob b and a, the coefficient of correlation being -0.987.

On the other hand, if only the smaller area is considered, there is no apparent community of intersection

points, even in rough approximation. Moreover, if the unit of measure is changed so that the lowest value of x (basilar length) in the data becomes $x = 1$, and the new values of log b are calculated, the relationship becomes appreciably less distinct. The coefficient of correlation now drops to – 0.887, which is significantly lower than the original value. A further increase in the unit of measurement would produce an even greater relative decrease in the coefficient of correlation.

It is clear, therefore, that the existence of a well-defined relationship between the constants of the relative growth curves is in this instance adequately explained by two factors: first, that the curves are required by the size limits of the data to pass through a restricted area; second, that the values of b are made to fall a considerable distance outside of this area. In fact, a relationship would be obtained for any set of curves selected at random, which conform to these two conditions. Furthermore, since these conditions usually obtain in practice, it is not surprising that equation (2) is so frequently found to be applicable.

The statement of Lumer (1939b) that an inverse relationship arises from the fact that a is dimensionally contained in b is therefore true only in a very limited sense.[1] What his analysis proves is merely that where a relationship occurs it will tend to be exponential in character.

[1] The equation given by Lumer (1939, p. 341) as expressing the manner in which b varies with changing units of measurement is incorrect. The correct equation is derived as follows:

In the original unit of measure we have

$$y = b_o x^{\alpha}.$$

If, for this unit, we substitute a new one which is q times as large, the values of x and y become x' and y', where

$$x = qx' \text{ and } y = qy'.$$

Then the original equation becomes

$$qy' = b_o (qx')\alpha,$$

and the new value of b is given by

$$b = b_o\, q^{\alpha - 1}.$$

The equation given by Lumer, on the other hand, is

$$b = b_o\, q^{1-\alpha}.$$

The constant α is thus contained in b as a positive, not a negative exponent.

The foregoing considerations show that, under ordinary circumstances, b and a will usually prove to be related aside from any question of dimensionality. The only general exception is the case in which the relative growth curves are parallel when plotted on a double logarithmic grid, *i.e.*, in which a remains fixed while b varies.

The coordinates of the theoretical point of intersection, as obtained from the constants of equation (2), may be regarded as representing the average values of the coordinates of the actual intersection points. In a situation such as that discussed above, this point will in general fall at about the center of the range of the data (see, for example, Fig. 2). Here there would appear to be no particular value in determining its coordinates, since these would tell us nothing useful about the curves in question beyond what can be determined by inspection alone.

In those instances, however, in which an approximation to a common intersection point occurs, *i.e.*, in which the curves on the whole intersect within an area which is small compared to the total range, the theoretical point may have a special significance with reference to the particular set of data. Thus Lumer (1940), in an analysis of skull proportions in adults of a number of breeds of dogs, found that for some of the relationships investigated the curves tended to converge at their upper ends toward a point in the neighborhood of that representing the European wolf. He interpreted this as supporting the theory that the ancestral form of the domesticated dog is an animal closely resembling the wolf, the present-day breeds having arisen largely through selection at various times for smaller body size. Here the coordinates of the theoretical intersection point, which lies close to the point representing the wolf, may be regarded as probably indicating the approximate proportions of such an ancestral form.

A similar illustration is provided by the results of Anderson and Busch (1941). They amputated the dorsal ramus of the antenna of *Daphnia magna* at different

levels of the first segment, and studied the subsequent growth of the segment in relation to total length. They found that most of the relative growth curves, together with the curve for unoperated animals, intersected in the neighborhood of the theoretical point, which fell at a value of body length of approximately five millimeters. This value, which is about the maximum length ever attained by animals of this species, here represents the size at which regeneration of the antennal segment would be complete. In other words, these results show that (within certain limits) the course of regeneration is such that it tends toward completion at roughly the same body size regardless of level of injury, the abscissa of the theoretical intersection point giving us the average value of this body size.

In these two examples, the particular sets of relative growth curves intersect in the upper portion of the range; that is, with increasing body size the organisms become more alike in their proportions. In this case the curves may be said to exhibit *convergence,* while the opposite situation, in which the point of intersection falls near the lower end of the range, may be designated as *divergence.* Where the curves intersect at an intermediate point they may be regarded as exhibiting convergence followed by divergence. In any of these three cases, however, the theoretical point of intersection represents the particular point at which body proportions are most nearly alike.

It is possible, of course, that a group of organisms may show convergence with respect to one structure and divergence with respect to another. This raises the question of what relationship might exist among the intersection points obtained for different structures, to which, however, no answer can be given at present.

The Biological Significance of the Constant b

The numerical values of b (or log b) and a are especially useful as a means of comparing growth relations in different forms or groups. The value of log b represents

a particular fixed point on the log-log graph of equation (1); hence, when two such values are compared, it is the difference in position of the two points (or the difference between the two values of log y when log $x = 0$) which is being examined. When, as is frequently true in practice, the unit of measurement is such that the point representing log b is far removed from the range of the data, such a direct comparison may yield entirely misleading results. This arises from the fact that the effect is to introduce a considerable extrapolation, so that the points compared do not lie within the range over which the equation can legitimately be considered as applicable.

This is well illustrated in the investigation of Lumer and Schultz (1941). In comparing postnatal relative growth in *Macaca mulatta* and *Macaca philippinensis,* it was found that statistically significant differences in both log b and a occurred in the upper arm/trunk, hand/trunk and tail/trunk relations. Moreover, the differences were in all three cases of approximately the same magnitude. Yet the graphs of these relations showed that whereas in the first two instances the curves for the two species almost overlapped, in the third they were a considerable distance apart. In other words, whereas the relative lengths of upper arm and hand are nearly alike in the two species, the relative tail length in *M. philippinensis* is much greater than that in *M. mulatta,* a distinction which is not disclosed by the differences in log b.

This discrepancy arises from the fact that, regardless of the relative positions of the curves within the range of the data, at the point where trunk height would be equal to one millimeter they happen to be approximately equidistant. Since the postnatal relative growth curves are not valid for this value of trunk height, the calculated values of log b corresponding to it are obviously meaningless from a biological point of view.

If the measurements are converted from millimeters to decimeters, the point at which $x = 1$ then falls near the value of trunk height at birth. The differences in log b

are now greatly changed. For the upper arm/trunk and hand/trunk relations they are decreased to the point of becoming insignificant;[2] for the tail/trunk relation, on the other hand, the difference becomes much larger. These new differences present more nearly a true picture of the actual variations in relative growth in the two species.

These considerations suggest that an even more appropriate value of b would be obtained by taking the value of x at birth as unity. The constant b would then be the size of the particular structure at the time of birth, and would thus represent the same physiological stage of development in all the species being compared. This would amount to selecting, in place of the conventional units of measure, a biological unit, given by the organism, a procedure similar to that adopted by Brody (1927) in his studies of growth age equivalence in different mammals.

It is evident from the foregoing that the seeming impossibility of attributing any biological significance to the value of b in so many instances is due to the obscuring effect of expressing this constant in terms of conventional units. This obscuring effect can be removed by selecting a unit determined by the data themselves. The most satisfactory unit would be the size of a standard part at the beginning of the developmental period to which a given set of equations apply. It is true that in some cases such a unit would not be readily ascertainable; here perhaps an approach might be made by a more arbitrary procedure, such as taking the smallest value of the standard part in the data as unity or selecting a conventional unit which would approximate it. This is aptly illustrated by Anderson and Busch (1941) in their study of allometry in normal and regenerating antennal segments in Daphnia where they chose the millimeter as the unit of measure and the animals were approximately one

[2] It should be noted that whereas the standard error of a, like the constant itself, is not altered by a change in units, the standard error of log b varies with the unit employed. This variation is obviously to be expected, since the sampling error of different ordinates on a curve varies from point to point, in a manner depending on the character of the particular data.

millimeter in length during the first instar. It may be noted that this would serve also to eliminate the arbitrary features of the relation between b and a which have been discussed in the preceding section. Of course, in the case in which the relative growth curves are parallel when plotted on a double logarithmic grid (all having the same value of a) differences in log b would not be affected by any change of units.

At any rate, it is clear that numerical values of b obtained in conventional units can not be uncritically used. If different groups of organisms are to be compared at a particular point in their development, which is what is involved when particular values of b are compared, it is necessary that the point selected must actually fall within the range of development and, further, that it must represent the same stage of development in all cases.

SUMMARY

The constants b and a of the law of allometry frequently are found to be inversely related for a given set of relative growth curves, which means that the curves in question intersect approximately at a common point. Since the numerical value of b depends on the units of measurement employed, the relationship between the constants can be arbitrarily varied (by selecting suitable units), so as to vary from inverse to direct, or *vice versa,* for the same set of data.

It is shown further that the existence of a relationship between b and a in many cases arises simply from the requirement, made by the data, that the relative growth curves pass through a restricted area, coupled with a choice of units which places the value of b some distance outside this area.

In addition, where b lies considerably beyond the range of the data, comparisons of this constant for different curves may be misleading. These obscuring arbitrary factors can best be ruled out by expressing b in terms of a unit given by the data, such as the size of a standard

part at a particular developmental stage, or at least in terms of a unit which places b within the lower limit of the range.

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AN INTERGENERIC HYBRID RATTLESNAKE[1]

DR. REEVE M. BAILEY
IOWA STATE COLLEGE

ALTHOUGH interspecific and intergeneric hybrids are well known in certain groups of vertebrates, especially fresh-water fishes, hybridization of distinct species of snakes is apparently an extremely rare phenomenon (Blanchard, 1921: 210; Vellard, 1929; Werner, 1936). It is therefore of interest to report a specimen believed to be an intergeneric hybrid rattlesnake, *Crotalus horridus horridus* Linnaeus×*Sistrurus catenatus catenatus* (Rafinesque). Dr. Howard K. Gloyd, of the Chicago Academy of Sciences, and Laurence M. Klauber, of the San Diego Society of Natural History, have examined the specimen and offered valuable suggestions in the preparation of this paper. I am obligated to Mr. Klauber for permitting me the use of certain pertinent information from his unpublished investigations, and to Dr. A. M. Lucas, of Iowa State College, for reading the manuscript.

The snake, now deposited in the Chicago Academy of Sciences, number 7815, was included in a collection secured from the Keokuk, Iowa, High School. The bottle containing the specimen bore no data. However, a careful consideration of the data on the other bottles and the species represented in the collection, and contact with the collector's daughter reveal that the entire collection originated in the immediate vicinity of Keokuk, Lee County, Iowa. All the material was collected prior to or about 1895 by the late Mr. C. F. Davis.

Zoogeographic and ecologic conditions at this locality apparently constitute no barrier to hybridization of *C. h. horridus* and *S. c. catenatus*. The Davis collection contained several typical examples of *horridus*. Although no individuals of *catenatus* were included, there is a specimen of this species in the Des Moines Historical Society

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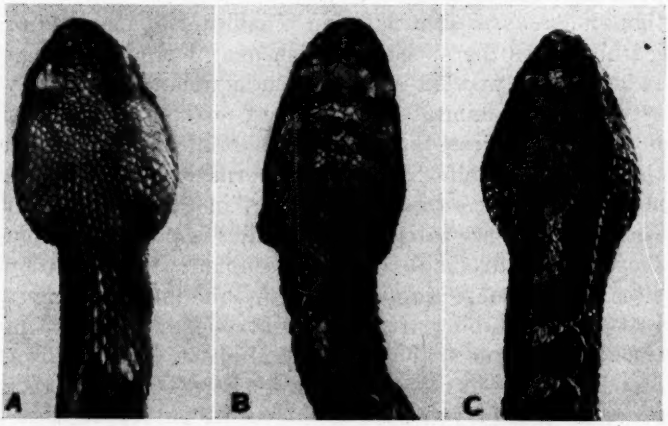
collection from Lee County, another in the Iowa State College collection taken 4 miles east of Farmington, Lee County, and Gloyd (1940: 50) has seen one from Burlington in the adjacent Des Moines County. The range of no other rattlesnake closely approaches Lee County, Iowa (Gloyd, 1940). It therefore appears that if the rattlesnake in question is truly a hybrid the parents are *Crotalus h. horridus* and *Sistrurus c. catenatus*. Timbered river bluffs, the habitat of *horridus*, are in proximity to the flood plain marshes of the Mississippi, where it is presumed *catenatus* lives or did live until recently. Although *catenatus* is usually an inhabitant of marshes or low meadows the specimen taken near Farmington was in a largely wooded area of rough upland, seemingly typical of the habitat preference of *horridus*. Whether or not this represents the normal habitat of *catenatus* in the area, it is quite likely that hibernation quarters are shared, or are contiguous, and that in the fall or after spring emergence interspecific mating is possible. That these species are at times joint occupants in hibernation sites is not mere supposition. While investigating a rattlesnake denning area along a wooded, rocky bluff of the Middle River near Winterset, Madison County, Iowa, on May 4, 1941, the author saw a *horridus* in a rock crevice. A hook was used to extract it, whereupon it became evident there were two snakes; one was a *horridus*, the other *catenatus*.

Description

The presumed hybrid is a female, 876 mm in total length to base of rattle, tail length 63 mm or 7.2 per cent. of total length. Head length 37.5 mm; head width 23.5 mm, not appreciably narrower posteriorly (as in *catenatus*); width of proximal rattle 11.6 mm; and fang length (Klauber, 1939: 20) 7.4 mm. Scale rows 27, 25, 23, 21, 19, with 23 along midportion of body; all strongly keeled except the lowest lateral row, which is smooth. Ventrals 163, subcaudals 24 (the last divided), supralabials 12–13, infralabials 14.

Rostral slightly higher than wide, truncate above, the edges flaring outward at about the middle and roughly parallel below. Nasals 2 on each side, the prenasal longer and in direct contact with rostral and first supralabial. Preoculars 2, the upper in rather broad contact with postnasal and the lower separated from postnasal by a single, large, subsemicircular loreal. Anterior to the maxillary pit and below the loreal and postnatal there are 4 small scales (3 on right side). Lacrimal of moderate size, separated from the fourth and fifth supralabials by a scale.



Photographs by Max E. Davis.

FIG. 1. Comparative dorsal head views of two species of rattlesnakes and a hybrid between them. A. *Crotalus horridus horridus:* a specimen 731 mm in total length taken near Winterset, Madison County, Iowa. B. *Crotalus h. horridus* × *Sistrurus c. catenatus:* the 876 mm specimen from Keokuk, Lee County, Iowa. C. *Sistrurus c. catenatus:* a 578 mm specimen from near Afton, Union County, Iowa.

Postoculars (counting all scales between lacrimal and supraocular) 4, the anteriormost separated from supralabials by 2 rows of scales. Upper temporal scales faintly keeled, lower smooth.

There are 2 normally formed internasals and 2 prefrontals, each of which is partially divided from its posterior edge. The frontal is divided into several scales (Fig. 1, B). The largest portion (which is asymmetrical)

is separated from the prefrontals by 3 irregularly shaped scales and from the left supraocular by one of these in addition to another small scale, but there is broad contact with the right supraocular. A somewhat smaller scale lies directly behind this largest remnant of the frontal; it is separated from the supraoculars by a scale on each side. A line connecting the posterior tips of the supraoculars crosses about 7 scales. The parietals are divided into many small plates, but the scales of this region are larger than those on the posterior third of the head, unlike *C. h. horridus* in this regard.

Coloration

The ground color of the back (in preservation) is light; the scales are finely flecked or stippled with dark, tending to approach *horridus* more nearly than *catenatus* in this respect. Along the dorsal mid-line there is a series of 31 (to vent) blackish-brown blotches, each clearly margined with black; anteriorly these are roughly quadrate in form, concave anteriorly and about 4 scales in length and 9 in width; posteriorly they are 2½ or 3 scales in length and are confluent with the midlateral blotches to form fairly regular crossbands which are widest at the midline (Fig. 2, A). The ratio between the size of the blotches and the interspaces more nearly resembles *horridus* than *catenatus*. There are 3 series of lateral blotches; those of the lowest are dark, the middle light-centered like those of the mid-dorsal series, and the uppermost faint and diffuse. The ventral surface is dark, profusely mottled and spotted with light. The tail is darker than the body color with 4 evident crossbands.

The neck bands, unlike those of *horridus* or *catenatus*, extend forward on the head to a point equidistant from the posterior end of the mandible and the anterior margin of the eye (Fig. 1, B); these markings are less sharply outlined anteriorly than in *catenatus* but more so than in *horridus*. Just anterior to each neck band there is a circular, dark spot about equal to the diameter of the eye;

between them there is a small median dark spot, and anterior to it are 2 larger paired spots which fuse at the midline (these are spaced about as are the dark spots of *horridus,* but are larger and more closely approximated). A semicircular dark bar extends from each eye mediad across the posterior third of the supraoculars and bends forward along their median edges, but these do not meet. The top of the head from the anterior third of the supraoculars forward is largely dark. A broad, sharply delimited, dark stripe which extends obliquely backward and downward from the eye to the side of the neck includes the last supralabial; the 2 supralabials anterior to it are clear white. The dark bar which extends downward from the pit includes all of the third supralabial and the anterior edge of the fourth, clearly intermediate in width between the narrow bar in *horridus* and the wide one in *catenatus.* From the posterior half of the fourth supralabial to the ninth the labials are light, heavily stippled with dark, giving a dusky or grayish appearance (Fig. 2, B), which is intermediate between the dark color in *catenatus* and the white or pale yellow in *horridus.* There is considerable dark pigment on the lower jaw, especially on infralabials 3 to 5 and 8 to 10, and in lengthwise streaks extending backward from the outer edges of the chin shields. The postocular stripe does not curve forward around the angle of the mouth onto the lower jaw as in *horridus.*

COMPARISONS

In order to facilitate comparison of this snake with *catenatus* and *horridus* they are contrasted in 24 differentiating characteristics in Table I, and the arrangement of scales and the color pattern of the top of the head are shown for each in Fig. 1, A, B, C.

The ventral scale count, 163, is of particular importance in the determination of this odd specimen, since in rattlesnakes it has been demonstrated (Klauber, 1937: 36) that the coefficient of variation seldom exceeds 2.5 per cent., and is generally less than 2 per cent. in homogene-

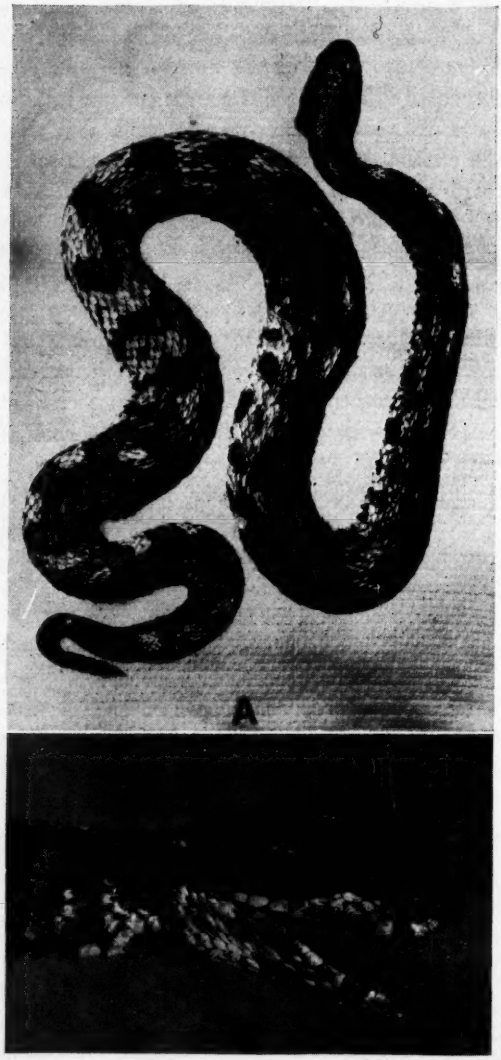
TABLE I

CONTRASTING CHARACTERS* OF *Sistrurus c. catenatus, Crotalus h. horridus* AND A HYBRID BETWEEN THESE TWO SPECIES

Character	*Sistrurus c. catenatus*	Hybrid	*Crotalus h. horridus*
Ventrals	Average 142 Range 136 to 151	163	Average 171 Range 164 to 177
Subcaudals	Average 23 Range 20 to 29	24	Average 20 Range 17 to 24
Scale rows at mid-body	25 (usually)	23	23 (usually)
Supralabials	(10) 12 (14)	12–13	(10) 12 to 15 (17)
Infralabials	(10) 12 or 13 (15)	14	(11) 14 to 16 (19)
Scales anterior to supraoculars	4	4	Average 16 Range 4 to 35
Minimum scales between supraoculars	1 (frontal rarely divided)	2	Average 6.4 Range 3 to 10
Parietal scales	Normally 2; greatly enlarged	Numerous; much larger than in *horridus*	Numerous; very small
Upper preocular in contact with postnasal	Almost 100 per cent.	Yes	5.8 per cent.
Loreals	1	1	2 (in 83 per cent.)
Scales between orbit and supralabials	1	2	Usually 2 or 3
Tail length/total length	Average .084 Range .070 to .098	.072	Average .062 Range .040 to .077
Fang length (mm)	About 6.5†	7.4	About 8.0†
Head length (mm)	About 35.7†	37.5	About 37.5†
Proximal rattle width (mm)	About 10.3†	11.6	About 13.0†
Number mid-dorsal body blotches	Average 33 Range 24 to 39	31	Average 24 Range 18 to 31
Form mid-dorsal body blotches	Roughly quadrate anteriorly, tending to fuse with mid-lateral blotches posteriorly; light-centered	Intermediate in shape; light-centered	Irregular anteriorly; forming chevron—shaped crossbands posteriorly; usually not light-centered
Lateral blotches	3 rows (upper faint and diffuse)	3 rows (upper faint and diffuse)	1 row
Tail color	Typically similar to body; 4 to 7 (mean 5.4) bands clearly evident	Darker than body; 4 bands evident	Typically almost black; 3 or 4 bands often faintly visible
Neck bands	Extend forward nearly to supraoculars; sharply outlined anteriorly	Intermediate in extent and sharpness of anterior edge	Scarcely entering head above posterior end of mandible; not sharply outlined anteriorly
Coloration of parietal region	Brown, with a large irregular median blotch, broadest anteriorly	Intermediate; see figure and description	Yellow, with a pair of small dark spots
Transverse dark bars on posterior portion of supraoculars	Present, usually connected at midline	Present, not joined at midline	Absent
Dark bar below maxillary pit	Broad; more than twice width of third supralabial	Intermediate; somewhat wider than third supralabial	Narrow; about half width of third supralabial
Supralabials below eye	Dark	Dusky	Light

* The characters of the two species are taken largely from Gloyd (1940) and Klauber (1936), and are those of females if sexual dimorphism is apparent. Gloyd's study included data on 348 specimens of *horridus* (190 females) and 341 of *catenatus*.

† Data provided by L. M. Klauber; theoretical expectancies for a snake of this size calculated from empirical regression curves.



Photographs by Max E. Davis.

FIG. 2. A hybrid rattlesnake, *Crotalus h. horridus* × *Sistrurus c. catenatus*. A. Dorsal view. B. Lateral view of head.

ous material. Compared with females of *catenatus* and *horridus* the hybrid is intermediate between the extreme counts recorded (maximum in *catenatus* 151; minimum in *horridus* 164). Mr. Klauber informs me that the ventral counts of 179 *catenatus* females average 142.407; standard deviation 3.522. Therefore this specimen is 5.84 standard deviations above the mean for *catenatus*, a figure of very high significance. The standard deviation for 211 *horridus* females is 3.077, mean 171.441, and the presumed hybrid is 2.74 standard deviations below the mean.

The total length, 876 mm, is unusual if the snake is considered *catenatus*. A female of size comparable to the largest recorded male *catenatus* (935 mm) would measure about 840 mm (computation by Klauber). Judging from the shape of the rattles this snake is not fully grown.

The proximal rattle of the hybrid measures 11.6 mm. The largest *catenatus* measured by Mr. Klauber (male, 935 mm) had a rattle 10.0 mm in width, and he has computed (using certain average regressions and extrapolations from the curve) that a female *catenatus* 876 mm in length would be expected to have a proximal rattle width of about 10.3 mm, whereas that of a female *horridus* would measure about 13.0 mm. In this character the specimen is clearly intermediate.

Discussion

It is apparent from these comparisons: (1) that the characters of scutellation, body proportions and color pattern of this snake are not distinctive (as would be expected if it were a third species), but are those of *catenatus* or *horridus* or, as in most features, variously intermediate between them, (2) that this specimen does not seem identifiable as either *C. h. horridus* or *S. c. catenatus* even after consideration of the possibility that it represents a highly variable and abnormal individual; such variants customarily diverge in one or a very few respects from the normal, not in many independent char-

acters. The hybrid is more or less intermediate between the species in 16 of the 24 characters tabulated; it agrees closely with *horridus* in 3 and with *catenatus* in 5 (4 of these 8 characters are of such a nature that intermediacy is impossible). The aberrant ventral scale count and the striking intermediacy in dorsal scutellation of the head and in practically all features of color pattern are especially notable. If we pronounce the specimen *catenatus* it deviates (with more or less significance) from the modal or typical condition in 19 of 24 characteristics considered; if judged *horridus* it varies in 21. In an animal so variable it is highly significant that if determined as either species all appreciable variations from the mode for that form are in the direction of the other species, and are not distributed at random as would be expected if there were no extraneous governing factor.

The frequent hybridization of fishes is a well-documented natural phenomenon, and has been verified by laboratory rearing. It has been demonstrated (Bailey and Lagler, 1938; Hubbs, 1940) that hybrid centrarchids and poeciliids are structurally intermediate between the parental species in most of their characters, and evidence derived from the study of hybrids in other groups of vertebrates adds weight to the conviction that morphological differences between vertebrate species in nature are dependent for the most part upon multiple Mendelian factors rather than on a single pair. Hence, the assumption that a snake which is structurally intermediate between two associated species is an interspecific hybrid seems justified.

In conclusion, since this snake does not represent a third species, seems improperly admitted to either *Crotalus h. horridus* or *Sistrurus c. catenatus,* is intermediate in most characters between these two, and since the two species are known to occur to the exclusion of other rattlesnakes at the locality of capture, it is interpreted as a hybrid between them.[2]

[2] The interpretation of this specimen as a hybrid is that of the author. After examination Dr. Gloyd was inclined to identify it as an aberrant *S. c.*

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catenatus. Mr. Klauber writes "... while I do not think it is possible to be certain that this is a hybrid, it is at least a very queer specimen indeed. I do not think I ever saw a specimen of any species which differed so widely from the mode of that species in so many independent characters. It seems to me that there are almost as many deviations when you consider the specimen to be *Sistrurus catenatus catenatus,* as if you reverse the process and consider it *Crotalus horridus horridus,* and then determine the number of characters in which it differs from the mode of that species."

A PHOTOPERIODISM ACCOMPANYING AUTOTETRAPLOIDY[1]

DR. J. G. O'MARA
U. S. DEPARTMENT OF AGRICULTURE, COLUMBIA, MISSOURI

INTRODUCTION

VARIOUS morphological differences between diploid organisms and their autotetraploid descendants or sibs have been described. These differences are generally in the same direction within a species, the tetraploid being coarser than the diploid and often larger (Winkler, 1916; Wettstein, 1924; Belling and Blakeslee, 1924; Lindstrom and Koos, 1931). Physiological differences, although as numerous and important as the morphological ones, are usually not discovered without special methods. The present report is concerned with a photoperiodism which affects the fertility of autotetraploid *Secale cereale.*

HISTORICAL

The slower growth of autotetraploids seems to be common to all species. This retarded rate was first noted by De Vries (1906) in *Oenothera Lamarckiana,* in which the gigas form has a tendency to behave as a biennial when *Lamarckiana* itself is annual. Reduced growth was also observed by Gates (1915), who determined that a difference in cell size existed between *Lamarckiana* and its gigas variant. The slower rate of growth was explained by Gates as the result of an altered ratio between cell surface and cell volume. The greater frost susceptibility of the tetraploid flowers was also observed by the same author. Nilsson (1920), using the same species, reported that the slower rate of growth of the gigas plant was also a characteristic of its pollen tubes. Stomps (1925), in a related species, *O. biennis,* noted that the gigas form developed more slowly than the related diploid form.

[1] This study is a part of the program supported by funds obtained under Bankhead-Jones Project SRF–2–5, "Comparative Genetics and Cytology of Polyploidy Series in Triticum," Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Missouri Agricultural Experiment Station, cooperating.

This same phenomenon has been demonstrated in numerous other genera. Keeble (1912) stated that tetraploid *Primula sinensis* is slower to mature than the diploid. In these respects *Solanum lycopersicum* is perhaps the most thoroughly investigated species. Kostoff and Kendall (1934) determined the nature of the growth of the diploid and autotetraploid by measuring the plants at thirty-day intervals. The retardation characteristic of other autotetraploids was found. The data and observations demonstrated that the retardation extended throughout the life of the plant. Jørgensen (1928) reported that tetraploid seeds required from three to five additional days to germinate. Lesley and Lesley (1930) confirmed the slower rate and were able to make further observations on it. Diploid root tips were found which had tetraploid islands in their meristematic tissue. Since the root tip meristem was symmetrical, the authors concluded that the larger tetraploid cells must have divided slower than the smaller diploid cells. Such islands also occurred in anthers of diploid plants. In this event, the tetraploid cells were at an earlier meiotic phase than the adjacent diploid microsporocytes.

Triploids may be admitted into these considerations, in that they differ from the diploid in the direction of the tetraploid. Nawashin (1929) observed that triploid *Crepis capillaris* developed slower than did diploid. Collins (1933) reported that triploid varieties of ananas matured more slowly than diploid ones.

In the lower plants, also, differences in growth rate are found in autopolyploid series. Dörries-Rüger (1929) studied the growth rates of various races of *Physcomitrium piriforme* and *Funaria hygrometrica.* In *Physcomitrium* a series of four members was available—from univalens to tetravalens, inclusive. The highest rate was that of the bivalens race, although the bivalens condition is not the natural one. These data seem to indicate that the diploid condition is the optimum one, physiologically, and that it has advantages not wholly genetic in nature. In these experiments the races were genetically identical.

The bivalens race of *Funaria* also grew faster than the univalens.

Differences other than those in rates of growth and maturity also occur. Wettstein (1924) reported that the univalens and bivalens races in various species of mosses reacted differently to various ion concentrations in their substrata. In the fern, *Polypodium aureum,* Heilbronn (1928) observed that the rate of starch formation was nearly twice as great in the tetraploid as in the diploid. In *Solanum lycopersicum* Sansome and Zilva (1933) found that autotetraploids had nearly twice as much vitamin C as the diploids. In a more intensive investigation of this species, Kostoff and Axamitnaja (1935) found that the tetraploids had less ash and more nitrogen, proteins, starch and water than the parental diploids. Golubinskij (1937) reported that an autotetraploid of *Ocimum canum* had more camphor than its diploid sib. Randolph and Hand (1938) analyzed diploid and tetraploid yellow *Zea mays.* The tetraploid had 43 per cent. more carotenoid pigment than the diploid. When the larger size of the tetraploid cells is considered, this increase proves to be a five-fold increase of carotenoid pigment per cell. Ruttle and Nebel (1939) reported that the odor of a tetraploid Mentha hybrid differed from that of the diploid. From an analysis of diploid and tetraploid *Lolium perenne,* Sullivan and Myers (1939) determined that the tetraploids had more reducing sugars, sucrose and total sugars than the diploids, and they also had proportionately more dry matter soluble in 80 per cent. alcohol.

More relevant to the present investigation, however, are the observations of Dorsey (1936) on autotetraploids of *Triticum vulgare,* varieties Honor and Forward. These autotetraploids did not flower when normal diploids flowered and set seed. When given long days, these tetraploids were induced to flower but were wholly sterile.

MATERIALS AND METHODS

The materials involved in the present experiment comprise (1) a diploid strain of *Secale cereale* with spring

habit; (2) the autotetraploid strain derived directly from this diploid; (3) *Triticum vulgare* var. Chinese, and (4) an amphidiploid of *T. vulgare* var. Chinese and a strain of *S. cereale* unrelated to the other *S. cereale*. The autotetraploid was kindly given to the writer in 1937 by Dr. Karl Sax, who also contributed the parental diploid for purposes of comparison when the tetraploid proved to be of interest. The amphidiploid was secured from Dr. E. R. Sears, and it and its parents have been described and illustrated by the writer (1940).

Experimental

In the fall of 1938 three plants of autotetraploid *Secale cereale* were planted in the greenhouse. These plants flowered in mid-December, shedding pollen abundantly. The anthers were much larger than those of an unrelated diploid strain which was flowering simultaneously. This increase in size was in accord with the other characters of the plant, which were generally those described as gigas. No seed occurred on these plants during the following few weeks. An examination of the pollen revealed that it was somewhat irregular in size and shape and that some unfilled grains were present. Most of the pollen, however, was filled and, although larger than diploid pollen, much of it was normal in appearance. Since *S. cereale* normally is self-sterile, hand pollinations were made among the plants to insure adequate compatible pollination. No seed was formed, and the plants were set aside as sterile. New sterile tillers continued to form throughout the winter, each one being removed as soon as its sterility was certain. In April a few seeds were detected, and thereafter seed was secured on most of the tillers. Plants grown in the field, from seed planted early in April, set seed on the first few tillers in varying low numbers, from one to fifteen. A few tillers had no seed, but these occurred on plants which had seed in most of the other heads. The most obvious explanation of these data would seem to be a photoperiodism involving fertility. In the following year this possibility was tested.

In the fall of 1939 thirty greenhouse plants of the autotetraploid were separated at random into two lots of fifteen plants each. One group was grown with natural day length, while the other was grown with seventeen-hour days, which were supplied with the aid of a 200-watt Mazda lamp. The groups were close together, being separated by approximately ten feet, a distance which seemed adequate for shielding the control group from the light above the treated group. The plants, started on September 7, shed pollen at approximately the same time in mid-December. On January 30 the heads were harvested. The group which had grown with natural days produced 49 heads, none of which had a seed. The group grown with long days produced 47 heads, 33 of which had seed. Both groups continued to produce new tillers, but no further record of fertility was kept.

The treated and therefore fertile plants were now used in a series of crosses in order to find a species with which the autotetraploid would cross. Such a cross would allow a test of the viability of the pollen or eggs or of both if it could be made reciprocally. The simplest method, that of reciprocal crosses between treated and untreated individuals, proved quite unsatisfactory, owing to the undependable fertility of the treated group and the abundance of pollen invariably about, since *Secale cereale* is an open-pollinated species. Crosses were made on diploid *S. cereale,* on *Triticum vulgare* var. Chinese, and on the amphidiploid of *T. vulgare* by *S. cereale.* The crosses involving diploid *S. cereale* and *T. vulgare* were remarkable in that every floret large enough to do so set a seed. These seeds proved, in every instance—over 300 in *S. cereale* and over 2,000 in *T. vulgare*—to be water seeds, which collapsed and dried to inviable shells after they had reached a normal size. The amphidiploid proved to be fertile with the autotetraploid pollen and so was selected for tests of the pollen in the following season.

In mid-February another planting of ten autotetraploids was made. These were allowed to grow under natural conditions to determine at what date the day

length was sufficient to induce seed-setting. The conclusion would apply only to the greenhouse conditions under which the material grew. The ten plants started to shed pollen on March 26, and the first seed was detected on April 25, after which seed occurred on most of the heads with the frequency characteristic of field plants.

In the course of these experiments, three plants out of 65 occurred which set no seed under long days and abundant pollination. In these instances another type of sterility must have occurred. Since the tetraploid is meiotically unstable, this sterility could have been the result of genome unbalance. A field-grown population of 34 plants was examined for chromosome number at meiosis. The chromosome associations are so variable that little can be concluded from them, and so the total chromosome number alone was recorded.

Chromosome No.	27	28	29	30
No. of plants	5	25	3	1

The 30-chromosome plant had some normal microsporocytes and some in which fragmentation had occurred to such an extent as to produce hundreds of chromatin pieces free in the cytoplasm. This plant was completely sterile. It is probable that excessive duplication or deficiency could produce similar sterile plants regardless of day length. Such deficiency and duplication could occur even within the euploid number.

In the fall of 1940 another thirty autotetraploid plants were separated at random into two equal groups which were grown at natural and seventeen-hour days. The results confirmed those of the preceding years. None of the fifteen unilluminated plants had seed when thirteen of the illuminated ones did have it. The pollen of the sterile group was now tested on the Triticale amphidiploid. Seven autotetraploid plants, selected at random from the sterile group, were used as pollen parents. All seven produced seed on the amphidiploid in a much higher percentage than the pollen of the amphidiploid when allowed to effect natural self-pollinations in the greenhouse.

In Table 1 are presented the results of these pollinations.

TABLE 1

POLLINATIONS OF A TRITICUM VULGARE-SECALE CEREALE AMPHIDIPLOID WITH POLLEN OF AUTOTETRAPLOID S. CEREALE GROWN WITH SHORT DAYS

Plant number	Florets pollinated	Seed obtained
1	30	11
2	56	21
3	30	9
4	28	11
5	28	11
6	58	28
7	30	6

The diploid strain from which the autotetraploid was derived was available for comparison with the illuminated and unilluminated groups. It was planted simultaneously with the autotetraploid and allowed to grow with natural days. It shed pollen two days before the illuminated group and seven days before the unilluminated group. Under natural days it was fully as fertile in the greenhouse in December as in the field in June.

From these data the conclusions seem necessary that the sterility of this autotetraploid constitutes a photoperiodic phenomenon directly dependent on the autotetraploidy, and that the sterility is egg- or zygotic-sterility and not pollen-sterility. These conclusions are based on the fertility of the parental diploid under short days and the viability of the pollen from sterile plants when placed on Triticale stigmas.

SUMMARY

Autotetraploid *Secale cereale* reacted to short days in a manner different from that of the diploid strain from which it was derived. The diploid was fertile throughout the year; the autotetraploid was female sterile under the natural short days of December and January. This photoperiodism constitutes another instance of a character present in an autotetraploid which is apparently unpredictable from the parental diploid.

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CARTILAGE AN EMBRYONIC ADAPTATION

DR. ALFRED S. ROMER
THE BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY

IT has been universally believed and taught that cartilage is the most ancient of vertebrate skeletal structures, and that bone is a later development which has replaced it during the evolution of the more advanced vertebrate groups. This belief has seemed firmly established on grounds both of phylogeny and ontogeny. The lowest of living vertebrates—cyclostomes and sharks—have purely cartilaginous skeletons, while bone is found only in the more advanced fishes and in land animals. In the embryos of bony vertebrates superficial bony elements may develop directly, but much of the internal skeleton is first formed in cartilage, which later is replaced by bone. Evolutionary and developmental histories thus seem to tell the same story; ontogeny appears to recapitulate phylogeny, and there is seemingly ample reason to justify current belief in the primitiveness of cartilage.

It is, however, highly probable that this pretty picture is a delusion and the reverse of the true situation. Modern evidence suggests that bone, not cartilage, was the primitive skeletal material; that cartilage originally was not an adult tissue but a purely embryonic one, evolved in connection with the development of internal skeletal elements; and that the presence of cartilage in the adult is indicative not of a primitive condition but of paedogenesis, the retention in the adult of an embryonic stage of the skeletal development.

It is true that the lowest of living vertebrates are boneless, cartilaginous forms. During the past two decades, however, much new evidence has accumulated concerning Paleozoic fossil vertebrates which shows the phylogenetic picture in a quite different light. It is now quite clear, and generally accepted by workers in the field, that bone is a very ancient tissue, for it was well developed and

universally present in the oldest known vertebrate faunas of the Silurian and Devonian periods. But while this fact is of interest, it does not in itself prove that bone is the older tissue. One might still maintain (although with difficulty) that this oldest known phase of vertebrate history was preceded by a still older one in which cartilage alone was present. In view of this possibility it is important that we should attempt to discover what the *trend* in skeletal evolution has been during the known history of the vertebrates. If there had been an earlier cartilaginous phase, we would expect that the trend would show an increased emphasis on bone in later forms. However, when the evidence is reviewed broadly, the opposite is seen to be the case. Almost universally there has been a trend not toward, but away from bone—a reduction in ossification and frequently an increase in the amount of cartilage present in the adult. This strongly indicates that bone was the primitive tissue, not cartilage, for it is difficult to believe (as one must if maintaining the primitiveness of cartilage) that the evolutionary processes underwent such a sharp reversal of direction.

The evidence indicating this trend may be briefly reviewed:

(1) The Class Agnatha, the jawless vertebrates, survives to-day only in the cyclostomes, in which the skeleton is purely cartilaginous. In the Silurian and Devonian, however, the group was represented by a host of varied forms commonly termed ostracoderms. The structure of many of them is now rather fully known through the work of Stensiö and other European workers. These ostracoderms had skeletons of bone, not merely in the form of dermal armor but also in many known cases, of ossified internal skeletal structures as well. The types present are highly diversified—some, for example, appear to be closely related to the lampreys, others show no resemblance to either lampreys or hagfishes. Because of this diversity it is impossible to believe, as was once suggested, that the ostracoderms form a single osseous side

branch of a primitive cartilaginous agnathous stock. One is forced to conclude that the ostracoderms themselves are the basal agnathous stock and that the modern cartilaginous lampreys and hagfishes are their descendants, as degenerate in their skeletons as they admittedly are in other features. Even within the known history of the Silurian and Devonian Agnatha can be seen the beginnings of this degenerative process, for Stensiö (1932, p. 27) has noted that the later genera are less ossified than the earlier ones.

(2) The sharks and their chimaeroid relatives, constituting the Class Chondrichthyes, are the lowest of living gnathostomes. Although hard parts may be present in the form of teeth, skin denticles and spines, their skeletons are entirely cartilaginous in both living and fossil forms.[1] It is thus but natural that sharks have been regarded as representing the primitive stock of jaw-bearing fishes in which bone had never developed.

This, however, is not the full story; for sharks are a relatively late group and are preceded both in time and in structure by a primitive class of gnathostomes now entirely extinct—the Placodermi.[2]

Sharks first occur in Upper Devonian strata. They are thus actually the last major group of fishes to appear in the geological record. Even the higher bony fishes were earlier, for they were abundant by the Middle Devonian and some were already present in early Devonian times. Still earlier were the placoderms, for they appear to have originated in the Silurian, flourished greatly in the Devonian and became extinct before the end of the Paleozoic.

The best known placoderms are the armored arthrodires and the "spiny sharks" or acanthodians. All were

[1] It is, however, of interest that Holmgren (1940, pp. 247–252) has noted condensations of mesenchyme in shark embryos corresponding to the pattern of dermal skull bones in other fishes.

[2] Watson (1937) in his recent valuable discussion of these forms, has coined for them the descriptive term Aphetohyoidea; however, Placodermi is a name of long standing and has the additional merit of brevity.

bony fishes, but despite this were (as Watson has pointed out) on a distinctly lower grade than the sharks. The first gill slit, for example, was fully developed and the hyoid arch unspecialized, in contrast with the reduced spiracular slit and specialized hyomandibular found in sharks and more advanced fishes. It seems obvious that the ancestors of the sharks passed through the placoderm stage. The better known placoderms appear to be too specialized to be these ancestors, but there are a number of more obscure types which may be annectant forms. Since the ancestral group possessed in all known instances a bony skeleton, it is reasonable to conclude that the present cartilaginous condition seen in sharks is the result of degeneration from the ancestral placoderm state.[3]

(3) The ray-finned fishes or Actinopterygii, whose modern representatives, the teleosts, are the dominant fish of modern days, were represented in the Paleozoic by the palaeoniscids. In them we find a highly developed bony skeleton which included both a complete external covering of dermal bones and thick ganoid scales and an internal skeleton almost entirely composed of bone. The fate of the skeleton in their descendants has been a varied one. (a) Both external and internal bony skeletons have been retained with little modification in the garpike and in the African genera *Polypterus* and *Calamoichthys*.[4] (b) The teleosts have retained (and even added to) the ossified internal skeleton; on the other hand, the thick bony scales of the ancestral forms have been reduced to relatively feeble structures or even lost entirely. (c) The sturgeon and paddle fish (*Polyodon*) are extremely de-

[3] The placoderms are in general too poorly known or too short-lived (geologically) to show within the group much evidence of a phyletic trend with regard to skeletal structures. Heintz (1929, pp. 20–21, 24–25, etc.), however, has presented evidence showing that in the arthrodires there was a gradual reduction of armor during the Devonian. As regards skeletal degeneration in the Chondrichthyes, see Woodward, 1931, pp. 5–6, and Stensiö, 1925, p. 188.

[4] These two genera are apparently little modified descendants of the palaeoniscids, and definitely not crossopterygians, as was long believed to be the case.

generate. The dermal armor is reduced and modified in the former; in the latter it has almost completely disappeared. The internal skeleton has nearly completely changed from a bony to a cartilaginous condition. With slight further reduction these forms would be as completely cartilaginous as the sharks, and one would be inclined to believe that they were primitive cartilaginous types. The evidence, however, clearly shows that these "cartilaginous ganoids" have descended through transitional Mesozoic types from well-ossified Paleozoic ancestors.

(4) The lungfish and crossopterygians form a closely knit group related to the ancestry of the amphibians; I have termed them collectively the Choanichthyes.[5] Both subgroups began in the Devonian and subsequently show clear evidences of skeletal degeneration. The lungfish show a progressive reduction of the dermal skeleton and a concomitant reduction of the degree of ossification of the internal skeleton; modern forms have retained little of the bone originally present. Among the crossopterygians the typical early representatives were well ossified; in the coelacanths, which alone of crossopterygians survived beyond the end of the Paleozoic, degeneration of both dermal and internal bone is strongly marked.

(5) In the Amphibia it is generally acknowledged that there has been a history of progressive skeletal degeneration. The Carboniferous forms were, as far as known, very well ossified internally, although dermal armor was already undergoing reduction. In the Permian and Triassic we witness a "progressive chondrification" (to use Case's term). In modern forms bony scales have disappeared, a majority of the dermal bones have vanished, and ossification of the internal skeleton is reduced.

[5] Dr. C. L. Hubbs has pointed out to me that he long ago proposed the term Amphibioidei for this same assemblage (1919, p. 570), while Camp and Vanderhoof in their recent bibliography of vertebrate paleontology have adopted the term Herpetichthyes, originally proposed by Huxley (1880, p. 660) to include all advanced fishes, but used in the present sense by Woodward (1931, pp. 8, 9).

(6) In the reptiles and other amniotes descended from them the internal skeleton retains its bony nature with little or no reduction—this retention seems obviously associated with the necessity of support in terrestrial life. The dermal bony skeleton, on the other hand, tends to be strongly reduced—except for sporadic instances the general covering of bony scales has vanished and dermal bones tend to be much reduced in numbers.

The review of skeletal history given above shows, I believe, quite clearly that the known history of vertebrates has been one in which in general bone has tended to be reduced and cartilage in a majority of groups has tended to grow in importance. It thus seems reasonable to conclude not merely that bone is an ancient tissue but also that it was the typical skeletal tissue of the adult ancestral vertebrate, and that the presence of cartilage in the adult skeleton is a secondary condition.

It will be noted that skeletal degeneration has been most extreme in the lowest groups and progressively less marked, on the whole, in the higher classes. Thus as regards the skeleton, the vertebrate "family tree" may be visualized as having a pyramidal tree form, with the lowest branches the longest—*i.e.*, departing most widely from the central trunk of bony types. From this it is easy to see how our current and, I believe, mistaken ideas of skeletal phylogeny have arisen. The living forms are, in this analogy, the leaves and twigs at the ends of the branches. When we study successively an ascending series of living vertebrates we are not, as we have fondly believed, viewing the trunk of the tree; on the contrary, we are observing a series of peripheral twigs which are successively less and less distant from the trunk (*i.e.*, typical primitive conditions) as we pass upward to the top. The true picture is thus the reverse of the assumed situation.

But even though the fossil record strongly indicates that bone is primitive, how are we to explain the supposed "proof" of the primitiveness of cartilage derived from

embryology—the fact that in the internal skeleton the elements are first formed in cartilage, and then later replaced by bone, giving a seemingly fine demonstration of recapitulation?

Opinions regarding the classic recapitulation theory have varied from implicit acceptance to complete rejection. Apparently the truth lies between these extremes. Vertebrates are conservative in their ontogeny, and many features of the embryo appear to be repetitions of ancient developmental processes. On the other hand, numerous structural characters of embryos are obviously adaptations to the exigencies of embryonic existence and have no phylogenetic significance. For example, no one believes that the ancestral amniote walked about with a caul over its head because of the universal presence of extra-embryonic membranes in the higher vertebrates; for these membranes are obviously adaptive features highly useful in the development of the egg of a land vertebrate. In the present instance, since the evidence strongly suggests that the development of cartilage in the embryo is not a repetition of any phylogenetic story, we are likewise justified in seeking a functional explanation of the presence of cartilage as the embryonic basis for adult bones.[6]

A clue to such a functional interpretation is afforded by the location of embryonic cartilages. Bones are in general readily classified into two types—dermal and endochondral. The former are situated in the skin and develop directly from mesenchyme condensations without an intervening cartilaginous stage. Bone, once deposited, is an inflexible material, but this character does not normally interfere with the direct development of superficial elements. Dermal bones are in general plate-like structures of simple form and without intimate connections with other organs. In consequence their development is without complication; once a tiny rudiment of

[6] Jaekel long ago suggested (1902, p. 1088) that cartilage might be accounted for in this fashion; but, largely because of lack of adequate knowledge of fossils of the lower vertebrates at this time, this suggestion received no consideration.

the plate is established, it may indefinitely increase the area it covers by simple accretions of bone at its margins, and since it "floats free" in the skin new layers of bone may be added to either inner or outer surfaces. It is thus possible for a superficial element to develop directly as a bone without the aid of any other type of formative material.

In strong contrast with this situation is that which we find in skeletal elements lying deeper in the body, as in the braincase, vertebral column and limbs. These are not simple plates but typically have a complicated structure with varied processes and rami which attain their characteristic shapes at an early and tiny stage. Further, they are in general intimately connected with one another in an architectural pattern established in the early embryo. Still further, they tend to assume, equally early, close relationships with other organ systems.

For example, in the human embryo the femur is well formed at a time when the entire embryo is but an inch and a half or so long. It has a shape closely approaching that of the adult femur. It has well-formed articular surfaces connecting with the girdle on the one hand and to tibia and fibula on the other. It has, further, a whole series of muscular attachments which are essentially those of the adult. At this stage the femur measures little more than an eighth of an inch in length. To reach adult conditions it must increase its length more than 100 times and its bulk many thousands of times. This growth can be accomplished only under definite restrictions: its terminal articular surfaces must continue unimpaired; its various processes must remain intact although shifted far proximally or distally; and its muscular attachments must be undisturbed despite a similar redistribution over the surface of the growing element.

It is obviously impossible to imagine the mode of growth of such a structure as the femur if it were formed of bone from the beginning. Bone is an unyielding material, incapable of expansion. It can grow only by the

addition of new layers on its surface; it can change its form only by such additions or by the reverse process of resorbtion. If so formed, the femur could increase in length only by the deposition of bone at its ends; this would result in continued disruption of its articular relations. It could shift its trochanters and condyles along the lengthening shaft to their definitive positions only by a complicated process of surface accretions and resorbtions which, while theoretically possible, is so unusual and complicated a process as to call for remarks when discovered in exceptional cases (*cf.* Davenport 1941, p. 464); this process would result in severing the muscular attachments made on these processes at an early period.

Bone, therefore, can be utilized in the longitudinal growth of such an element only in the final stages; some other type of material is needed in the embryo to function during early growth. The primary requisite for such a material is that it must be able to increase in size without disturbance of its surface relations; it must be able to grow internally by expansion.

This quality is, of course, the most marked feature of cartilage as a skeletal tissue. Cartilage is far inferior to bone in most regards as a protective or supporting material. But on the other hand it possesses the potentiality of growth by expansion. In contrast with bone, its cells can divide, and the daughter cells push apart in the surrounding matrix with the deposition of new accretions of cartilage between them. A cartilage thus, unlike bone, can and does grow by internal expansion, without the disturbance of surface conditions present in the case of bone growth. Some of the increase in size of cartilages may be due to the superficial addition of new materials; but in great measure growth appears to take place internally rather than at the surface.

In higher vertebrates bony centers form in the internal elements rather early in development, the relative amount of cartilage present at any later time is small, and the importance of this material tends to be minimized. Ac-

tually, however, the cartilage present shows a remarkable degree of expansion.

We may use again the example of the human femur. Ossification develops in the shaft at an early stage—the seventh week of intra-uterine life. From this stage on the cartilage is confined to relatively short areas at either end; shortly after birth there remain only thin rings between the bone of the shaft and the terminal epiphyseal ossifications. During these later stages of growth the cartilage appears, superficially, to be an inert and relatively unimportant part of the growing femur. Actually, however, this is the reverse of the true situation. The growth in the length of the femur is due entirely to the activity and continual growth of the cartilage. At the beginning the cartilages at either end of the shaft were but a fraction of an inch away from the mid-point of the bone; by the time adult size is reached they are more than a foot apart. This separation has been attained solely by their own growth and expansion. Each of the two tiny nubbins of the embryo has produced, mainly by internal growth, a mass of cartilage eight inches in length and with a volume of several cubic inches. The lack of apparent growth is of course due to the fact that the osseous tissue continuously takes advantage of this expansion, replaces the cartilage as rapidly as it develops, and deposits in perichondral fashion along the shaft thus formed, where articulations are absent and muscular attachments unimportant. Formation of adult bone is made possible only by the expansion of the embryonic cartilages which precede it.

The presence of cartilage as an antecedent to bone in development can thus be interpreted in a satisfactory manner as due to the ideal nature of this material as an embryonic adaptation for the formation of internal skeletal elements. The ontogeny of the oldest vertebrates is as yet unknown and may remain forever unknown. We can, however, be fairly sure that although the adults of these ancient forms were probably bony types, cartilage

or some related substance must have been present in their growth stages as a necessary aid to development of internal osseous structures. Cartilage, thus, is an ancient skeletal material, probably as old as bone. But primitively, we may believe, it was present only in the embryo and growing young. In later times it appears to an increasing extent in the adult stage in many vertebrate groups. Such a condition is not, as we have seen, to be regarded as a primitive one in a phylogenetic sense. On the contrary, it is evidence of a trend toward the retention of an embryonic condition in the skeleton of the sexually mature animal. Adult cartilage is an indication of neoteny.

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CHROMATOPHORES AS EVIDENCE OF PHYLOGENETIC EVOLUTION[1]

DR. HANS ELIAS
MIDDLESEX UNIVERSITY

IN this society, where generally physiological experiments are chiefly discussed, a morphological theme may also be of some interest. Melanophores are known to you as branched, pigmented cells which have the ability to contract and expand in response to certain stimuli. Especially here in Cambridge much has been done by, and under the leadership of Dr. G. H. Parker to clarify their physiological properties and to show the nature of the agents and stimuli which act upon them. These stimuli have been called *melanokins* by Bytinski-Salz (1938), because they produce kinesis of the melanophores. Their function is the regulation of color in many animals, among which I will consider only the Amphibians, in which group the melanophores undergo an interesting change during phylogenetic evolution.

In amphibians we find four types of melanophores classified according to their location: (1) inside the epidermal epithelium (epidermal melanophores); (2) immediately under the epidermis (adepidermal melanophores); (3) in the corium, but very close to the epidermis (intracutaneous melanophores); (4) under the corium (subcutaneous melanophores). The second and third types of melanophores formerly had the common name of subepidermal melanophores, but since they are of a different nature, I introduced in 1936 the above-mentioned names.

In Urodeles we find two kinds of melanophores in larvae and adults: namely, epidermal and adepidermal melanophores. Both types are controlled by melanokin stimuli: namely, temperature, humidity, light, hormones and certain pharmacological agents.

Fig. 1 shows adepidermal melanophores in a recently hatched larva of Ambystoma. They are arranged nearly

[1] Presented before the Boston Society of Biologists, February 14, 1940.

parallel and have a thread-like appearance. From this first stage they develop in urodeles to the highly branched, flat form as in Fig. 2. (Epidermal melanophores are also present here).

In the tailless amphibians the development is different. I do not know their behavior in the most primitive Anura-family of *Liopelmidae,* but in *Discoglossidae,* a very primitive family, too, their development is as follows:

FIG. 1. Adepidermal melanophores in the tail of a just hatched larva of *Ambystoma mexicanum.* Magn. : 200 ×.

In *Discoglossus pictus* (Fig. 3) the adepidermal melanophores remain thread-shaped, grow in length and, growing along the inferior surface of the epidermis (which means within one plane), they meet one another and adhere together. However, as soon as one melanophore has reached another, further active growth in length ceases, and their further increase in length is still due only to the traction which one melanophore exercises upon its neighbor. The whole development of the structure, which this

network undergoes, is passive growth, produced by the extension of the growing epidermis, so that the structure of this network is the product of tractive and adhesive forces between the growing epidermis and the melanophores on the one side, and between the melanophores among each other on the other side, as I pointed out in 1939 (b) and 1941.

As every melanophore is now fixed at its ends, a con-

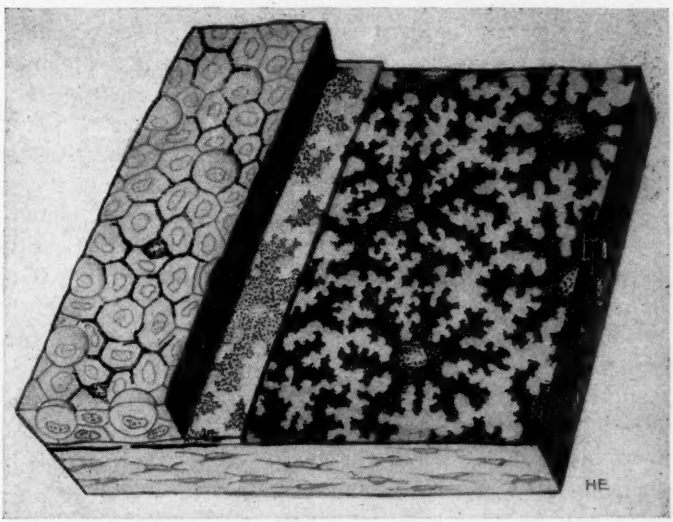

FIG. 2. Semi-diagram of the arrangement of melanophores in the Urodeles' skin (both larvae and adults).

traction is no longer possible, and it is probably therefore why the adepidermal melanophores in *Discoglossus* tadpoles have completely lost their contractility, as Bytinski-Salz and I showed in 1938 in regenerating tails. Even though the adepidermal melanophores are temporarily isolated, nevertheless they are not contractile.

The adepidermal melanophores in *Discoglossus* no longer have the general color-regulating properties of melanophores, and no melanokin has any influence upon them.

They become very thick and very long (up to 8 μ in diameter and 500 μ in length). Thus they form a kind of 2-dimensional, giant mesenchyme which may be seen even with the naked eye and which increases considerably the toughness of the skin. Last summer I had the opportunity to make experiments upon the mechanical properties of *Discoglossus* tadpoles. The skin was extended between two glass needles, one of which was flexible. The amount of curvature of this needle indicated the forces applied for various degrees of extension. To extend a piece of skin in places where no adepidermal melanophores are present to about 1½ times its original length, only as little forces as 7 to 20 milligrams are necessary. With further extension, however, the skin assumes a plastic or liquid consistency, so that it does not offer any resistance to further extension. The adepidermal melanophores, however, are elastic; *i.e.,* they offer a steadily growing resistance proportional to the degree of extension; and they contract to nearly their original length when the traction is released. The force necessary for their extension varies with the thickness of the melanophores, and it can be calculated that their modulus of elasticity is 190 kg/cm.^{2} This means that we would have to apply a force of 190 kg in order to extend a thread, made up of the same substance as the adepidermal melanophores, to double length, if that thread had a cross section of 1 cm.^{2}

We can conclude from this observation that the adepidermal melanophores have changed their function completely, the network they form having become a supporting organ.

An amphibian, however, needs flat, color-regulating melanophores. Thus a new type of flat, movable melanophores has been developed in *Discoglossus* tadpoles, with the same physiological properties as the adepidermal ones of the Urodeles: namely, the *subcutaneous melanophores* (Fig. 3).

Still the adepidermal melanophores give a slight con-

tribution to the design of the tadpole. They are no longer flat and movable, but have at least the appearance of black lines. In this respect they have become similar to the epidermal melanophores. The latter are therefore superfluous in young tadpoles and develop only in a late stage during metamorphosis when the adepidermal melano-

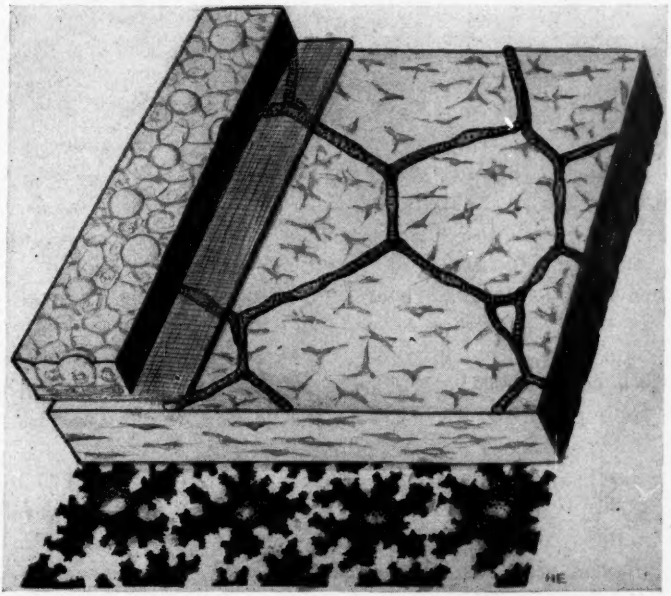

FIG. 3. Semi-diagram of the arrangement of melanophores in the skin of *Discoglossus pictus* tadpoles.

phores begin to degenerate. In young tadpoles they are absent.

In *Bombina,* the next higher member of the *Discoglossidae,* the adepidermal melanophores have assumed a slightly different form (Fig. 4). They are no longer adherent to one another, are T- or cross-shaped and are arranged to form a very regular, rectangular pattern. Their arrangement in this manner seems due to the fact that in any region of the body a straight, stiff thread can

maintain its shape better in the direction of maximum or minimum tension (usually perpendicular to one another), as in these two directions such a thread is not submitted to forces which would tend to bend it. Indeed, during early development the adepidermal melanophores send out spurs in every direction, but only those running in two definite directions, perpendicular to one another, per-

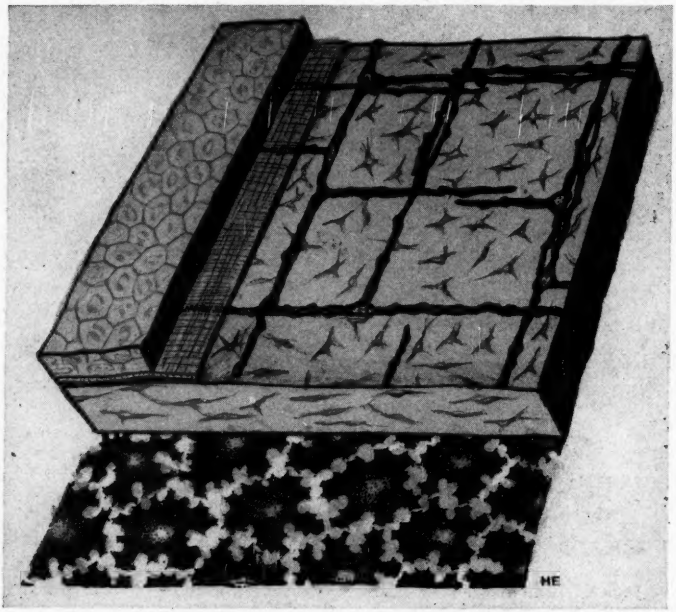

FIG. 4. Semi-diagram of the arrangement of melanophores in the skin of young tadpoles of *Bombina variegata* var. *pachypus*, *Alytes obstetricans* and *Pelodytes punctatus*.

sist, while the others disappear. As evidence for the assumption that melanophores are passively arranged in the direction of maximum tension the following experiment may serve: The adepidermal melanophores, when present—as it is the case in tadpoles with albinotic adepidermal melanophores—are oriented chiefly in the same direction as the latter. I implanted, for a reason which

I will consider later, frog's pituitary under the skin of tadpoles of *Bombina*. After 4½ hours all the epidermal melanophores were radially arranged around the graft. In order to find the reason I made a time lapse picture which shows that the wound over the graft is closed by a simple contraction of the skin, as it is done when a sack is closed. There is no active movement of the epidermal

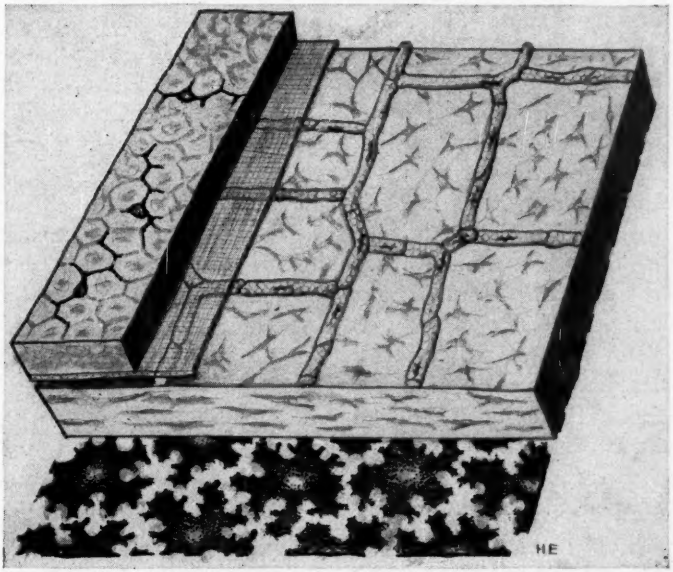

FIG. 5. Semi-diagram of the arrangement of melanophores in the skin of old tadpoles of *Alytes obstetricans* where the adepidermal melanophores are pigmentless, and in the semialbinotic generations of the Zürich variety of *Bombina variegata* var. *pachypus*.

melanophores, but the melanophores are passively oriented in the direction of the maximum tension. The same happens with the deeper situated adepidermal melanophores.

The epidermal melanophores of *Bombina* are also independent of the melanokins. And even extirpation of the pituitary anlage does not disturb the full development of

the adepidermal melanophore network, as shown in 1938 by Bytinski-Salz.

Also in *Bombina* no epidermal melanophores are present in young tadpoles. But a layer of subcutaneous melanophores which respond to melanokins is present.

In Zürich on two occasions, once in 1866 (Eberth) and once in my own observations in 1938 it had been observed that the adepidermal melanophores of *Bombina* tadpoles had no pigment (Fig. 5). These semialbinotic tadpoles were collected in the same pond in which in other years all the tadpoles had well-pigmented melanophores. I first ascribed this albinism to some disease, but their pigmentlessness could not be influenced in any way. None of these following means were able to produce pigmentation: Darkness, blinding, black ground, cold, feeding with pituitary in various forms, injection of pituitary extract, implantation of pituitary. Thus we have to conclude that this type of partial albinism is a genetical property of the animal. Curiously enough, in the same population in one year all the individuals had a normal pigmented net. During the next year, nearly all the individuals had unpigmented adepidermal melanophores. There seems to be an alternation of pigmented and pigmentless generations.

It seems to be necessary for tadpoles to possess thread-shaped melanophores in one layer of the skin or in another. For, we find epidermal melanophores in all those tadpoles where the adepidermal melanophores are pigmentless (Fig. 5).

We now come to what is generally regarded as the next higher species. The tadpoles of *Alytes obstetricans* have, as Prenant (1923) showed, when they are young, a well-pigmented adepidermal melanophore network of the same structure as we know it from *Bombina* (Fig. 4). But when they grow older, their pigment disappears, and they have the same appearance as the albinotic melanophores which we find in some generations of the Zürich population of *Bombina*. Every individual of *Alytes* thus exhibits, in two successive phases of its development, both states occurring in various generations of *Bombina*.

Pelodytes, which Clauss puts into the family of *Discoglossidae,* but which Noble considers already to be a toad, still possesses adepidermal melanophores of the same kind as *Bombina.*

In all the higher Anura, however, from toads to frogs, adepidermal melanophores are no longer present. Apparently the newly acquired network is not a necessity, neither as a pattern of black lines (because epidermal

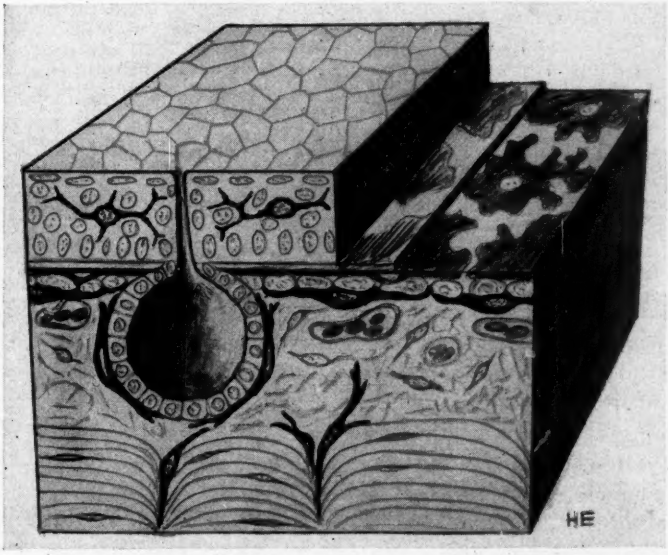

FIG. 6. Semi-diagram of the arrangement of chromatophores in the skin of toads and frogs.

melanophores still are available), nor as a mechanical support for the skin.

At the same time as adepidermal melanophores no longer have their original color-regulating function which has been assumed by the subcutaneous melanophores in tadpoles, a certain difficulty arises for the adult tailless Amphibians: While in tadpoles the subcutaneous melanophores are visible from outside because of the transpar-

ency of the skin, they are no longer visible in adults, since the derma of adult Anura becomes opaque. Thus during metamorphosis a fourth type of melanophores arises from connective tissue cells within the upper layer of the corium, the intracutaneous melanophores, as shown for various species by Elias (1931, 1934, 1936, 1937). And while in *Discoglossidae* the subcutaneous melanophores—although invisible—persist, they degenerate in toads and frogs during metamorphosis. There we find the conditions shown in Fig. 6.

Melanophores in Amphibians represent, as I attempted to demonstrate, a cytological example of the change of function of an organ during phylogenetic evolution.

In the first place it is replaced in its former function by two new organs; and in the second place, because its newly acquired function was not a vital necessity for the animal, it finally disappears.

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REVIEWS AND COMMENTS

EDITED BY CARL L. HUBBS

In this section reviews and notices are given of current publications on general biology and of specialized works which have an important bearing in this general field. Emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution.

REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as otherwise indicated, all items are prepared by the Section Editor, Dr. Carl L. Hubbs, University of Michigan, Ann Arbor, Michigan. All opinions are those of the reviewer.

Genes and Chromosomes Structure and Organization. Cold Spring Harbor Symposia on Quantitative Biology, 9, 1941: i–x, 1–315, 18 pls., 64 figs.

THE search for fundamentals in the mechanism of inheritance reaches a milestone in this symposium volume. The detail and diversity of treatment, the lack of clear-cut answers, reflect the present state of knowledge about genes and chromosomes. The variety of opinion would perhaps have been increased only by including Goldschmidt, who would, we suppose, have doubted the existence of genes.

The symposium leads off with four contributions on the cytology of chromosomes. Then five authors, including C. W. METZ, treat the chromosomes of the salivary glands. Six follow with papers on "Spontaneous and Induced Changes in Chromosome Structure," which are unwisely segregated as phenomena distinct from "Mutations." The gene mutations are reported on by five of the most outstanding of the many contributors, namely H. H. PLOUGH, M. M. RHOADES, M. DEMEREC, H. J. MULLER and L. G. STADLER. "Physical Aspects and Tools" include two items. The general feeling of the day that there is some kinship between virus and gene led to the inclusion of nine contributions, under the heading "Properties of Giant Molecules as Related to Chromosome Problems." The "Résumé and Perspectives of the Symposium on Genes and Chromosomes" is furnished by H. J. MULLER, who ends the large volume by expressing the thanks of the

contributors "to the organizers of this Symposium, for having for the first time focussed the efforts of so many specialists from very diverse fields upon that group of problems which is most fundamental for the understanding of the distinctive phenomena of life."

Mass Collections—The Technique and Use of Mass Collections in Plant Taxonomy. By EDGAR ANDERSON. Mass Collections: Camassia scilloides. By RALPH O. ERICKSON. Mass Collections: Rubus odoratus and R. parviflorus. By NORMAN C. FASSETT. Annals of the Missouri Botanical Garden, 28, 1941: 287–374, 3 figs., 36 maps, 5 pls. $1.25 (paper).

"MASS COLLECTIONS" are presented as a new tool in taxonomic researches on plants. The ordinary herbarium material is too selective for variants, to represent accurately the frequencies of variations, the discontinuity of variation and the correlations between variables. The method is that of adequate, randomized sampling—of the whole plant for small species and of critical parts for large ones. Essentially similar methods have been used for years in zoological collections, particularly of fishes.

The technique and use of mass herbarium collections are briefly presented in the introduction by Edgar Anderson, the able geneticist of the Missouri Botanical Gardens. There follow two examples of the use of the method, a short one by Ralph O. Erickson and a long one by Norman C. Fassett. These samples verify the claim that extensive randomized herbarium samples are of great value in the analysis of variation and speciation in plants. The fact that the material is preserved will make it possible for others to restudy problems.

The Rat in Laboratory Investigation. By a Staff of Thirty Contributors. Edited by JOHN Q. GRIFFITH, JR., and EDMOND J. FARRIS. Philadelphia: J. B. Lippincott Co., 1942: i–xvi, 1–488, figs. 1–177, pls. 1–2. $7.50.

THE albino rat is the real guinea-pig of scientific investigation. A great service was done to science when the inbred albino rats were made generally available for researches in almost all phases of biology, from anatomy to

pathology and psychology. An almost equal service is now given, in the form of a very thorough and authoritative treatment of the care and biology of this commonest of laboratory animals. Especial emphasis is given to methods of rearing and handling the rats and of employing them in a wide variety of investigations. Every laboratory using albino rats should have a copy of this book at hand.

Man and the Vertebrates. By ALFRED SHERWOOD ROMER. Chicago: University of Chicago Press, 1941: i–ix, 1–405, 417 figs. 3rd edition. $3.50.

THE enlarged and revised edition of "Man and the Vertebrates" is undeniably "comprehensive." Many will wonder how much a beginning student will likely absorb from so large a factual dose of vertebrate zoology, comparative anatomy, paleontology, and of anthropology, now including ethnology as well as archeology. In a skilful effort to make this concoction of disciplines acceptable and digestible, the pill has been sugar-coated. The diction is simplified and pepped up, without introducing a too-glaring mixture of the scientific and journalistic styles. The very copious illustrations are well chosen and obviously designed not only to render the technicalities clear but also to hold the interest of the student. The branches of biology emphasized in this survey text are well integrated. Professor Romer has succeeded remarkably well in his aim of presenting a "fairly comprehensive account of the evolution of the vertebrates and man with a minimum of technical minutiae," and the publishers have cooperated finely in producing a streamlined job.

The classification of the vertebrates (outlined and diagrammed in the appendices) is a mixture of old and new. Thus the immense agglomeration of the Teleostei is left in a single order, whereas the Crossopterygii and Dipnoi are combined under the subclass Choanichthyes. Incidentally this name is retained despite the fact that the group was earlier named Amphibioidei by the reviewer.

Ecology, economic zoology, comparative physiology, histology, cytology, genetics, eugenics and other branches of zoology are scarcely mentioned, and the embryology and physiology of the vertebrates are given comparatively little space in this book. No doubt many biologists would prefer to emphasize some of these subjects, believing them to be more vital to an appreciation of human biology than are the items that are expanded. But what a pill it would have been, had those subjects too been included!

Strange New World. The Adventures of John Gilbert and Ludwig Leichhart. By ALEC H. CHISHOLM. Sydney and London: Angus and Robertson, Ltd., 1941: i–xxiii, 1–382, 25 pls., 2 maps.

FIELD naturalists, ornithologists and historians will all find much of interest and value in this livid account of early exploration in Australia. The author beams with enthusiasm over the discovery in England of John Gilbert's diary of his expedition with Leichhart, the erratic German who became an Australian hero. The new evidence, confirming previously questioned data, clinchingly stamps Leichhart as poorly balanced, self-centered, inexperienced and incapable, outstandingly successful only in his unalterable ambition to become a great explorer and in his ability to induce others to finance his education and his trips. John Gilbert, on the other hand, is now snatched from obscurity, and pictured as a worthy personality, an able explorer and an accomplished field naturalist.

NOTICES OF NEW BOOKS

Ecological Crop Geography. By KARL H. W. KLAGES. New York: The Macmillan Co., 1942: i–xviii, 1–615, figs. 1–108. $4.50. —Geographical and ecological in its approach, this detailed monograph will be of great value to many biologists. The chapters on climatology give a good introduction to that subject. Extensive references are given to the literature, but we note the lack of any mention of the scholarly work of the Russian scientist, N. I. Vavilov, on the ecological control of evolution in cultivated plants.

The Beginnings of Social Behavior in Unicellular Organisms. By HERBERT S. JENNINGS. Philadelphia: University of Pennsylvania Press, 1941: 1–17. $0.25.—This Leidy Memorial Lecture is published as one of the series from the University of Pennsylvania Bicentennial Conference. It is a summary of the author's classical researches on incipient sociology as displayed by the Protozoa.

Tabular Keys for the Identification of the Woody Plants. By FLORENCE B. ROBINSON. Champaign, Illinois: The Garrard Press, 1941: i–ii, 1–156. $2.50.—A manual for the identification of the woody plants of the northern United States and Canada.

Plant Biology. By PAUL WEATHERWAX. Philadelphia and London: W. B. Saunders Co., 1942: i–vi, 1–455, figs. 1–182. $3.25.—This text is well written, thoroughly illustrated with original photographs and diagrams, and attractively printed. It provides a comprehensive introductory treatment of the subject. The various phases of botany are helpfully integrated, and there is balance between the topical and systematic approaches.

I. The Classification of the Genus Drosophila, with Descriptions of Nine New Species. By A. H. STURTEVANT. **II. New Species in the Quinaria Group of the Subgenus Drosophila.** By WARREN P. SPENCER. **III. Description of New Species of the Subgenera Hirtodrosophila and Drosophila.** By J. T. PATTERSON and MARSHALL R. WHEELER. University of Texas Publ., 4213, 1942: 1–109.—The tie-in of *Drosophila* research with speciation has been notably advanced by this cooperative systematic research. Such studies are breaking down the boundaries that have unfortunately developed between the biological subsciences.

The Photodynamic Action of Dyes on the Eggs of the Sea Urchin, Lytechinus variegatus. By DAVID HILT TENNENT. Washington: Carnegie Institution of Washington, Publ. 539 (Papers from the Tortugas Laboratory, Vol. 35), 1942: 1–153, figs. 1–40, pls. 1–8. $1.25 (paper), $1.75 (cloth).—"A fundamental contribution to the general fields of photodynamic action and the nature of the cell surface."

Boy of the Woods. The Story of John James Audubon. By MAIE LOUNSBURY WELLS AND DOROTHY FOX. New York: E. P. Dutton & Co., 1942: 1–142, 12 pls. (by Elinore Blaisdell). $2.00. —Though written in novel-style to catch the interest of children, this attractive little book gives an authoritative picture of the life of the most famous painter of birds.

The Soils that Support Us. By CHARLES E. KELLOGG. New York: The Macmillan Co., 1941: i–xi, 1–370, figs. 1–80. $3.50.—

"This is a simple book about soils and their relationship to people by one who loves them both. It is written for the general reader, the student, or the scientist, who needs to know something about the nature, use, and conservation of the soils."

ITEMS RECEIVED

Boletin del Instituto Botanico de la Universidad Central, Quito, Ecuador, Vol. 1, No. 1, 1942: 1–255.

Notes on a Collection of Fishes from Antigua and Barbados, British West Indies. By ALBERT W. C. T. HERRE. Stanford University Publications, Univ. Ser., Biol. Sci., 7, 1942: 285–305. $0.50 (paper), $1.25 (cloth).

Scientists Face the World of 1942. By KARL T. COMPTON. **The Case for Biological Engineering.** By VANNEVAR BUSH. **The Case for Agricultural Engineering.** By ROBERT W. TRULLINGER. **Commentaries.** By HARVEY N. DAVIS, DETLEV BRONK and S. W. FLETCHER. New Brunswick, N. J.: Rutgers University Press, 1942: 1–80. $1.25.

SHORTER ARTICLES AND DISCUSSION

THE DEVELOPMENT AND RELATIONSHIPS OF *GLYPHOCRANGON* (CRUSTACEA DECAPODA CARIDEA)

THE development of the Caridean family Glyphocrangonidae seems to have been discussed only by Bate (1888; p. 515; Pl. XCII, 4), who offers an undetailed description and figure of an embryo extracted from the egg of *Glyphocrangon granulosis*. This embryo is stated to have had "every appendage . . . present in a more or less advanced condition," and Bate surmises that it would develop further to hatch "in a very matured condition."

According to an examination of late embryos extracted from the eggs of *Glyphocrangon cf. spinicauda* A. M. E., it seems possible that this form may at hatching, although in the main highly precocious, be provided with well-developed exopodites on the first two pairs of legs. Limitation of exopods to the first one or two pairs of legs is especially characteristic of Crangonid larvae and this feature therefore tends to confirm the accepted view that there is a close relationship between Crangonidae and Glyphocrangonidae. The characters of the Glyphocrangonid embryo (taken from eggs *ca.* 3.2 mm by 2.4 mm in long and short axes) are as follows:

The cephalothorax, inflated with a very large yolk mass, and the pleon suggest in form those of the first stage of *Sclerocrangon ferox* (a Crangonid with abbreviated development, the first two hatched stages of which are maintained in the maternal brood-pouch) described by Wollebaek (1906). As in young *Sclerocrangon,* the soft integument of embryonic *Glyphocrangon* bears no indication of the vigorous adult sculpture, although there are rudiments of pleonic pleural angles, and there is a small subrectangular antennal angle on the carapace. The rostrum is a short, unarmed, subconical projection bent down to the frons between the eyes. The broad escutcheon-shaped telson differs strikingly in distal armature from that figured by Bate for his Glyphocrangonid embryo, as well as from that of Crangonid larvae. It bears a very small spine on the lateral side of the posterolateral angles, three somewhat longer slender spines upon the angles, and a row of 20 to 30 small spinules along the posterior margin on either side between the small but well-developed tri-

angular median prominence and the lateral angle. In earlier embryos the posterior margin of the telson is medially notched and the armature is limited to the posterolateral angles. The embryonic cuticle which encloses the developing telson shows no trace of a more normal spinulation.

The moderately large, unpigmented eyes are lightly soldered to the carapace. The proximal segment of the antennular peduncle bears no spine nor even so rudimentary a stylocerite as that of the adult; two short flagella are present. The antennal scale differs from the highly modified one of the adult in being essentially normal in form, subrectangular, with externo-distal spine near the tip at the end of a thickened external margin which is separated by a deep channel from the likewise thickened median part of the blade.

The mouthparts, although soft and unarmed, are essentially similar to those of the adult, the most significant difference being in the second maxilla where traces of two proximal endites are visible in addition to the very reduced distal one. The first maxillipede differs from that of the adult in having a large exopod like those of following limbs instead of a reduced and palp-like one. The endopod of the second maxillipede is unflexed; it resembles that of Wollebaek's young *Sclerocrangon* in that only the carpus is defined by discernible joints, the dactyl not being separated from the propodus. The endopod of the third maxillipede resembles in shape that of young *Sclerocrangon*, although it is entirely unarmed and the joints are ill-defined; its exopod is longer than the endopod.

The first two pairs of legs are provided with large fleshy exopods like those of the maxillipedes although smaller; that of the first leg reaches nearly to the tip of the propodus of the endopod, that of the second leg past the base of the carpus. The exopods bear well-developed setae under the naked embryonic molt-skin which encases them. The endopod of the first leg resembles in shape and size that of the third maxillipede but has a distinguishable rudiment of joint between the propodus and the conical, unflexed dactyl; the ischial spine of the adult is not indicated, nor the ischio-meral joint. The second leg has a small dactyl but no fixed finger; the carpal subjoints of the adult are not indicated. The posterior three pairs of pereiopods lack any trace of exopods; the endopods are large but with simple conical dactyls not provided with any rudiment of claws such as those by means of which

young *Sclerocrangon* are said to maintain themselves in the maternal incubatory chamber.

The pleopods are moderately large though not setose; the first pair has a very small endopod without appendix interna; the second through fifth have endopods nearly as large as the exopods, with conspicuous appendix interna. The uropods are small fleshy rudiments soldered to the telson in the manner usual in abbreviated development.

There are epipodites on the first two pairs of maxillipedes only, as in the adult. There is no trace of the arthrobranchs nor of the maxillipedal pleurobranch of the adult, but the five pleurobranchs above the pereiopods are large and well-developed.

It is difficult to judge precisely how near the described embryo may be to eclosion. However, the relatively high grade of development of the exopods of the first two pairs of legs suggests that these branches may possibly be functional after hatching, and that the form may pass through a period of quasi-larval life.

It seems of some interest in connection with the relationships of the Glyphocrangonidae, to consider the peculiar bathypelagic Caridean *Physetocaris* recently described by Chace (1940) as the type of a new family. According to its author, *Physetocaris* "apparently shows affinities with the Processidae and the Crangonidae" but "even its relative position among the established families is uncertain." However, the combination described by Chace for *Physetocaris* of non-chelate first pereiopods bearing "a sickle-shaped dactyl" with second pereiopods in which the carpus is multiarticulate, is otherwise found only in *Glyphocrangon;* and would at first glance, when added to the reduced mouthparts, suggest a relationship between the two genera. Of evidence against such relationship, although Chace states that the terminal segment of the second maxillipede of *Physetocaris* is "applied normally," "not . . . as a strip to the end of the preceding joint" (a feature which if correctly described would sharply distinguish *Physetocaris* from the Processidae, Crangonidae and Glyphocrangonidae), examination of his figure 63K suggests that his interpretation of this limb may be erroneous. The penultimate joint seems to be carpus, not propodus, so that the second maxillipede of *Physetocaris* appears merely to resemble that of the embryonic *Glyphocrangon* (and of various larval Caridea) in lacking an articulation between propodus and dactyl.

There are, however, other features described by Chace, in which *Physetocaris* differs conspicuously from the Crangonoida (and the

Processidae); these are that its rostrum is large and medially denticulate, that it lacks an exopodite on the first maxillipede, and that it bears epipodites on the third maxillipede and the first three pairs of legs. Consideration of this non-conformity leads to a critical reexamination of the first leg of *Physetocaris*, since this is the structure which would seem most suggestive of a Glyphocrangonid affinity. Chace's figure 63c of this limb indicates that it possesses but six segments. Such a reduction is not extremely unusual in Caridea, where the ischium of the first leg is sometimes fused with the merus; but when the ischium and merus are fused it is unusual for this compound segment to be much shorter than the carpus, as would be the case in *Physetocaris* if the terminal joint here is the dactyl. In fact, the form and proportion of the joints of the first leg of *Physetocaris* so closely resemble those in certain Pandalidae in which the dactyl is entirely absent (*Chlorotocoides*, DeMan, 1920, Pl. XV, Fig. 46; *Thalassocaris* post-larva, Gurney and Lebour, 1941, Fig. 13b) that it seems extremely probable that the terminal joint believed by Chace to be the dactyl is actually the propodus. In this case, the pereiopods of *Physetocaris* would not be of Processid or of Crangonoid type at all, but would be characteristically Pandaloid.

Comparing *Physetocaris* with Pandalidae, the arrangement of branchiae and exopodites seems to present some more or less unusual similarities (although these can be matched by Hippolytidae; compare *Paralatreutes* with the Pandalid series *Chlorotocoides, Chorotocella, Chlorocurtis;* Kemp, 1925, pp. 334, 280-1). The telson of *Physetocaris* is evidently not like that of adult Pandalidae and other Caridea, since spines are described at its tip only; but this is a character which like several others of this form might be retained from larval stages. The third maxillipede of *Physetocaris* seems to differ from that of other adult Caridae in the great length of the segment following the coxa (I am informed by Dr. Chace that the coxa of the third maxillipede, omitted from his figure, is separated by a distinct joint from the following segment). The segment following the coxa is in other adult Caridea the basis, a short article separated by a joint from the ischium which is fused with the merus; but in Caridean larvae the jointing of the third maxillipede may be quite different from that in the adult. According to Berkeley (Fig. 60, 1931), in the first stage larva of *Pandalus hypsinotus* the ischium is fused with the basis and distinct from the merus,

producing a relatively very long segment following the coxa; and persistence of such a larval character might account for the form of the limb in *Physetocaris*.[1]

In the reduction of its mouthparts, *Physetocaris* differs very conspicuously from the Pandalidae (in which it is only in *Chlorocurtis* Kemp that even the mandibular palp is absent); but a tendency toward reduction of these structures is widely and erratically distributed among Caridea (and it should be noted that Gurney, 1923, has produced evidence that *Processa* is probably not nearly related to the Crangonidae). In contrast, the loss of the dactyl of the first legs seems, aside from *Physetocaris*, to be limited to Pandalidae; and this character would suggest that Chace's form is tentatively to be regarded as a specialized Pandaloid rather than, as his description might at first glance be taken to indicate, a Crangonoid.

MARTIN D. BURKENROAD

BINGHAM OCEANOGRAPHIC LABORATORY,
YALE UNIVERSITY

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[1] The holotype of *Physetocaris* has been examined since the foregoing was written, through the kindness of Dr. William Beebe and Miss J. Crane. Among points supplementary to the original description it seems worth noting here that the ovary of the specimens is nearly ripe. There is what appears to be a trace of an articulation in the proximal part of the second segment from the base of the third maxillipede; and the articulation between the third and fourth segments from the base of the first leg is rigid in contrast to the movable articulation next distad; which observations seem to confirm the foregoing interpretations of limbjoint homology in this interesting probable Pandaloid.

THE INHERITANCE OF A SUBSPECIFIC CHARACTER IN THE *VIRILIS* COMPLEX OF DROSOPHILA

THE two subspecies or, as some workers prefer to call them, species, *Drosophila virilis virilis* Sturtevant and *Drosophila virilis americana* Spencer, produce fertile hybrids of both sexes in both of the reciprocal crosses.

Virilis differs from *americana* in a great many characters, but one of the most striking differences is that of the color of the pupa cases. The pupa cases of *virilis* are gray or black, those of *americana* mahogany red.[1]

The inheritance of this color difference was first studied by Spencer (1938), who found that the mahogany red color of the pupa cases in *americana* appears to be completely dominant, and the F_1 hybrid offspring of either of the reciprocal crosses between the two subspecies have pupa cases of the *americana* color in both sexes. Spencer further reports (1940) that all the morphological and physiological characters by which the two subspecies differ that are amenable to segregation studies depend on multiple factors and are not inherited as simple one-gene differences. However, he points out (written communication) the possibility that the color of the pupa cases may behave differently in different strains, as his preliminary results suggested simple mendelian inheritance of this character, while later work did not support this hypothesis.

In the course of crosses between *virilis* and *americana* carried out by the author in determining the chromosome homologies between the two subspecies (Stalker, 1940), pupae of back-cross progeny were isolated, and the genotypes of the emerging adults compared with the color of the pupa cases. From the data (unpublished) so obtained it seemed probable that the dominant gene or genes for red pupa case were carried on the fifth chromosome, with the autosomal V-shaped chromosome corresponding to 2 and 3 of *virilis* carrying supplementary genes for redness.

It should be mentioned at this point that the two subspecies differ markedly in their chromosome configuration. The work of Hughes (1939), Patterson, Stone and Griffen (1940b) and Stalker (1940) has shown that while the *virilis* metaphase plate shows one pair of micro-chromosomes and five pairs of rod-shaped

[1] Ridgeway's Color Standards were used in all references to the color of the pupa cases. Although the names are not exactly descriptive of the colors encountered, they give a means of comparison that may be useful.

chromosomes in both sexes, some of the *virilis* chromosomes appear in a fused condition in *americana*. The diagram below shows the nature of the fusions, (indicated thus: .....).

Drosophila virilis virilis						
female:	(X)	(4)	(2)	(3)	(5)	(6)
	(X)	(4)	(2)	(3)	(5)	(6)
male:	(X)	(4)	(2)	(3)	(5)	(6)
	(Y)	(4)	(2)	(3)	(5)	(6)
Drosophila virilis americana						
female:	(X.....4)		(2.....3)		(5)	(6)
	(X.....4)		(2.....3)		(5)	(6)
male:	(X.....4)		(2.....3)		(5)	(6)
	(Y)	(4)	(2.....3)		(5)	(6)

Thus genetical marking of chromosome 2 in a hybrid between the two subspecies gives information on the segregation of chromosome 3; and in certain cases the segregation of chromosome 4 may be determined from the sex of the progeny.

Shortly after the completion of this analysis, Patterson, Stone and Griffen (1940b), reported that the gene, or closely linked group of genes causing red pupa cases in *americana*, was carried on the arm of the autosomal V-shaped chromosome corresponding to chromosome 2 of *virilis*. These authors based their conclusions on cytological evidence, which, however, was not given in detail.

It seemed that the difference between the conclusion of these workers and those of the writer might be explained in a number of ways. First, the stock of *virilis* used in the two laboratories was not the same. Secondly, the *virilis* used in this laboratory contained an eye color mutant gene (varnished), and a body color gene (yellow), and the objection could have been raised that these genes might have had some effect on the color of the pupa cases. Finally, differences in culture medium might have been responsible.

The strain of *virilis* used in the Texas laboratory was the Pasadena strain, which according to the Texas workers had "tannish-gray" pupa cases. (Patterson, Stone and Griffen, 1940b, p. 219.) Although both the Texas stock, and that used by the writer came from Dr. W. P. Spencer, of Wooster, Ohio, and are presumably the same, the stock used in the experiments reported in this paper had black pupa cases. The reason for this difference may be that the culture medium used in the Texas laboratory is different from that used here. Dr. Sturtevant (written communication) informs me that in the Pasadena laboratory this stock of *virilis* also produces black pupa cases.

In order to obtain further data on the inheritance of the pupa case color, two additional sets of crosses were carried out between *virilis* and *americana*. This paper reports the results of these crosses.

EXPERIMENTAL

In both sets of crosses, the Smithville strain of *americana* (that used by the Texas workers) was used. In one case the Pasadena strain of *virilis* was used, in the other a mutant *virilis* strain which contained no mutant color genes. Males of both *virilis* stocks were crossed to *americana* females, and the F_1 hybrid males back-crossed to the *virilis* stocks. The same colors were found in the pupa cases of the back-cross progeny in both experiments. From this it was concluded that the color of the pupa cases was inherited in an essentially similar way in both the Pasadena wild-type and the mutant *virilis* strain.

The *virilis* mutant strain carried the genes for tiny bristles (tb) and gap^2 (gp^2) on the third chromosome, and ruffled (ru) and interrupted (i) on the fifth. The following crosses were made:

P_1 Smithville *americana* ♀♀ × tb gp^2; ru i (*virilis*) ♂♂
B. C. tb gp^2; ru i (*virilis*) ♀♀ × hybrid ♂♂

The pupae of the back-cross progeny were isolated and the phenotypes and the genotypes of the adults compared with the color of the pupa cases. Table 2 gives the results of these comparisons. Table 1 gives the colors of the pupa cases in the pure strains and the F_1 hybrids.

TABLE 1

COMPARISON OF CHROMOSOMAL CONSTITUTIONS AND COLORS OF PUPA CASES IN *Virilis*, *Americana* AND THEIR F, HYBRIDS

Strain or hybrid	Sex	*americana* chromosomes present	Color of pupa cases
Smithville *americana*	♂♂, ♀♀	full diploid set	mahogany red
Pasadena *virilis*	♂♂, ♀♀	none	black
tb gp^2; ru i *virilis*	♂♂, ♀♀	none	black
F_1 hybrids			
americana ♀♀ ×	♂♂	X..4 2..3 5 6	mahogany red
virilis ♂♂	♀♀	X..4 2..3 5 6	mahogany red
F_1 hybrids			
virilis ♀♀ ×	♂♂	Y 4 2..3 5 6	mahogany red
americana ♂♂	♀♀	X..4 2..3 5 6	mahogany red
F_1 hybrids			
americana ♀♀ ×	♂♂	X..4 2..3 5 6	mahogany red
tb gp^2; ru i ♂♂	♀♀	X..4 2..3 5 6	mahogany red

From Table 1 it can be seen that there was in no case any sexual difference noted in the colors of hybrid pupa cases. Thus

it would appear that neither the V-shaped *americana* X.....4, nor the sex-limited male 4 and Y carry any genes exerting a marked effect on the production of redness in the pupa cases. However, it might be concluded that the *americana* male carries red-producing gene(s) in the X.....4 chromosome and a similar gene or set of genes in the Y and/or the sex-limited male 4. In this case the F_1 (*virilis* × *americana*) hybrids of both sexes would receive red-producing genes from the *americana* father.

But Table 2 shows no differences between females which have the *americana* X.....4 and males which have *virilis* Y and 4. Thus apparently neither X.....4 nor Y and 4 of *americana* carry red-producing genes.

TABLE 2

COMPARISON OF CHROMOSOMAL CONSTITUTION AND COLORS OF PUPA CASES IN BACK-CROSS PROGENY OF tb gp²; ru i ♀♀ × HYBRID (*americana* ♀♀ × tb gp²; ru i ♂♂) ♂♂. CHROMOSOME 6 WAS NOT FOLLOWED IN THIS CROSS

Phenotypic class	Sex	*americana* chromosomes present	Pupa case color	No. of cases
tb gp²; ru i	♂♂	none	black	16
tb gp²; ru i	♀♀	X..,4	black	17
ru i	♂♂	2..3	black	25
ru i	♀♀	X..4 2..3	black	19
tb gp²	♂♂	5	bay	17
			mahogany red	2
tb gp²	♀♀	X..4 5	bay	17
			mahogany red	5
wild-type	♂♂	2..3 5	mahogany red	39
wild-type	♀♀	X..4 2..3 5	mahogany red	32

In Table 2, *americana* chromosome 6, being unmarked, can not be followed in any particular individual. However, it should be present in half of the members of all classes. But in the phenotypically tb gp²; ru i, as well as in the wild-type classes, all individuals have black, *virilis*-type pupa cases in the first case, and mahogany red, *americana*-type in the second. Thus chromosome 6 apparently carries no genes which exert any noticeable effect on coloration of the pupa cases.

In the phenotypically ru i class, the males carried only the V-shaped *americana* 2....3 autosome, while the females carried two *americana* V-shaped chromosomes; X....4 and 2.....3. However, the pupa cases were all black, as in *virilis*.

In the phenotypically tb gp² class, the females carried *americana* chromosomes X....4 and 5, while the males carried only *americana* chromosome 5. Since the pupa cases of both sexes

were distinctly reddish, although much darker than those of *americana,* we may conclude that *americana* chromosome 5 contains one or more dominant genes which, with or without the *americana* gene(s) in X....4, can produce distinctly reddish pupa cases. The colors in both sexes of this class ranged from Ridgeway's mahogany red to bay (bay has considerably more brown in it than mahogany red). Although intermediates between the two colors were found, the best determinations that could be made gave for the males, 17 bay and 2 mahogany red, and for the females, 17 bay and 5 mahogany red. This slight difference between the two sexes is hardly significant. Should it be confirmed in more extensive material it would suggest that in this particular genotypic milieu, chromosome X....4 exerts some effect in causing redness of the pupa cases.

In the phenotypically wild-type individuals, the pupa cases are mahogany red and indistinguishable from those of the F_1 hybrids and pure *americana.* In this class the females contain *americana* chromosomes X....4, 2....3 and 5, while the males have chromosomes 2....3 and 5. Thus *americana* chromosome 2 3 contains one or more dominant genes, which in conjunction with *americana* chromosome 5 produces mahogany red, *americana*-type pupa cases.

In summary, the two *americana* chromosomes 5 and 2....3 carry dominant red-producing genes. However, while the gene(s) on chromosome 5 are noticeably effective by themselves, the gene(s) on 2....3 produce no effect unless in the presence of *americana* chromosome 5. When both 2....3 and 5 are present, the former acts as an enhancer of the latter.

Thus these results remain in contrast to those of Patterson, Stone and Griffen (1940b). Our data show two linkage groups to be involved in the determination of the subspecific character.

HARRISON D. STALKER

UNIVERSITY OF ROCHESTER

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PINUS: EMBRYO SIZE COMPARED WITH GROWTH RATE

IN 1936–37 Professors Eric Ashby (1937 a and b) and E. M. East (1936) in separate papers debated the relationship of size of embryo to vigor in plants. Ashby had found experimentally that seeds which produced F_1 hybrids showing heterosis were heavier than seeds of the parent forms. He maintains that "Heredity controls plant size . . . chiefly through determining the initial size of the primordia," and explains his data on the basis of principal and interest; the greater the principal (primordial size) the greater the total accrued interest (plant size) at the same interest rate (rate of growth, which he found to be essentially constant between his plants).

Although the findings of E. M. East were not in accord with these, the implications of Ashby's work were of such weight that it was decided at the Institute of Forest Genetics to test the applicability of the findings to the pines, *P. Jeffreyi* and *P. Lambertiana* being chosen for the test because of their large seeds and availability.

In 1938, groups of pine seed (total 559) screened to standard size were pasted to white cards and photographed by x-rays according to directions very kindly supplied by the Eastman Kodak Company of Rochester, N. Y. Enlargements were made to facilitate measurement. No satisfactory technique was devised for measuring precisely the primordia as suggested by Ashby (1937a), but on the assumption (perhaps erroneous) that primordial size would be reflected in total embryo size, the following measurements were taken: Total length, length excluding cotyledons, width at point of cotyledon attachment and width of hypocotyl.

The seeds were planted in outdoor seedbeds at Placerville, Calif., and at the end of the first and the second growing seasons height and diameter measurements of the seedlings were taken. These were related with the corresponding embryo measurements,

and the resulting data were analyzed by the statistical section of this station.

No significant correlation was found between any of the embryo measurements and the relative size of the seedlings grown from them.

PALMER STOCKWELL

INSTITUTE OF FOREST GENETICS,
CALIFORNIA FOREST AND RANGE EXPERIMENT STATION[1]

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[1] Maintained by the U. S. Department of Agriculture at Berkeley, Calif., in cooperation with the University of California.

75-YEAR INDEX OF *THE AMERICAN NATURALIST*

A LETTER was sent to all subscribers to THE AMERICAN NATURALIST to learn if enough advanced orders could be obtained to compile and print a 75-Year Index to the Journal.

Only 220 orders at $6.00 per volume were received. This is not enough to pay even half of the expense.

Will those who can use the Index please let the editor know so that it might be possible to proceed with the compilation?

JAQUES CATTELL,
Editor